Lipases

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AA Arachidonic acid.
CLA Conjugated linoleic acid.
DAG Diacylglycerol.
DHA Docosahexaenoic acid.
DPA Docosapentaenoic acid.
EPA Eicosapentaenoic acid.
FA Fatty acid.
FFA Free fatty acid.
GCL Geotrichum candidum lipase.
GLA \(\gamma\)-Linolenic acid.
HPL Human pancreas lipase.
MAG Monoacylglycerol.
PUFA Polyunsaturated fatty acids.
RML Rhizopus miehei lipase.
SOS 1,3-Stearoyl-2-oleoyl glycerol.
TAG Triacylglycerol.
TAG-DDO TAG with 2 mol of docosahexaenoic acid and 1 mol of oleic acid.

Abbreviations

FDA Food and drug administration
TAG-DOO TAG with 1 mol of DHA and 2 mol of oleic acid
TAG-OOO TAG with 1 mol of DHA and 2 mol of triolein
VODD vegetable oil deodorizer distillates

Defining Statement

Lipases are enzymes that catalyze the hydrolysis of triacylglycerols (TAGs) to glycerol and fatty acids (FAs). Plants, animals, and microorganisms produce lipases. Animal lipases are found in the pancreas and in gastric processes. Microbial lipases are relatively stable and are capable of catalyzing a variety of reactions; they are potentially of importance for diverse industrial applications.

Introduction

Lipases (triacylglycerol hydrolases E.C. 3.1.1.3) are enzymes that catalyze the hydrolysis of triacylglycerols (TAGs) to glycerol and fatty acids (FAs). Lipase was first identified in the pancreas by J. Eberle in 1834 and by C. I. Bernard in 1856. Lipases, together with amylases and proteases, constitute the three major known digestive enzymes. However, because of the difficulty in handling its water-insoluble substrates and the heterogeneous reaction system, lipase was rarely in the main stream of research. Plants, animals, and microorganisms produce lipases. Animal lipases are found in several different organs, such as the pancreas and digestive tract. Earlier investigations were concerned mainly with enzymes that participated in lipid metabolism in animals. The most thoroughly studied was lipase from the pancreas. Recently, increasingly more attention is being paid to lipases produced by bacteria and fungi. Microbial lipases are relatively stable and are capable of catalyzing a number of reactions; they are potentially of importance for diverse industrial applications.

In recent years, information on the mechanistic properties of lipases has become available. The notion that lipases have a catalytic triad consisting of Ser-Asp-His was confirmed by the structures of the human pancreatic lipase (HPL) and Rhizomucor miehei lipase (RML). For the Geotrichum candidum lipase (GCL), the catalytic triad was...
found to be Ser-Glu-His. In all three cases, the side chains of the active site amino acids form a configuration that is stereochemically very similar to that of serine proteases. In contrast to the proteases, the lipases share the common feature that the active site is buried in the protein. In the case of the HPL and RML, the active site is covered by a short amphipathic helix or ‘lid’, whereas the active site of GCL seems to be covered by two nearly parallel amphipathic helices. The lid moves away upon interaction with the substrate. It has been proposed that this conformational change results in the activation of these enzymes at an oil–water interface. However, this interfacial activation phenomenon does not apply to all lipases. For example, lipases from *Pseudomonas aeruginosa* and *Candida antarctica* B do not have a ‘lid’, and therefore lack interfacial activation. These enzymes constitute a bridge between lipases and esterases. Accordingly, lipases can be defined as esterases that are able to catalyze the hydrolysis of long-chain triacylglycerides. Studies on lipase were accelerated in the 1980s when lipases were reported to be active and stable in water-immiscible organic solvents. Lipase was used as an ingredient in detergents, and an immobilized 1,3-position-specific lipase was applied for the industrial production of a cocoa butter substitute using a fixed-bed bioreactor. The use of lipases has been increasing steadily in the oil and fat industry.

**Substrate Specificity**

Substrate specificities of lipases are classified into FA specificity, positional specificity, alcohol specificity, TAG specificity, acylglycerol specificity, and stereo and chiral specificities. Because these specificities are changeable by different reaction conditions, much care is necessary when lipases are used as catalysts for the precise processing of oils and fats and for chiral resolutions.

**FA Specificity**

FA specificity of lipase is affected by the carbon length of the FA, and by the number and position of double bonds. In addition, FA specificity is changeable not only under different conditions but also in different reactions. For example, a lipase shows strong FA specificity in esterification with lauryl alcohol and 1-menthol compared with the hydrolysis of TAGs.

**Effect of carbon length**

Many lipases recognize C<sub>8</sub>–C<sub>24</sub> FAs and react strongly, but a group of lipases, such as *Candida rugosa* and *G. candidum* lipases, react weakly on C<sub>20</sub> or greater carbon length of FAs.

**Effect of number of double bonds**

Activity of *C. rugosa* and *G. candidum* lipases on C<sub>18</sub> FAs are in the order of stearic acid (18:0) < oleic acid (18:1ω–9) < linoleic acid (18:2ω–6) < α-linolenic acid (18:3ω–3). In general, lipases act weakly on polyunsaturated FAs (PUFAs), such as arachidonic acid (AA; 20:4ω–6), eicosapentaenoic acid (EPA; 20:5ω–3), and docosahexaenoic acid (DHA; 22:6ω–3).

**Effect of double-bond position**

Many lipases react weakly on FAs with double bonds near the carboxyl group. Lipases from microorganisms, such as *C. rugosa*, *G. candidum*, *R. oryzae*, and *R. miehei*, react strongly on α-linolenic acid carrying a double bond at Δ9, but weakly on γ-linolenic acid (GLA; 18:3ω–6) carrying a double bond at Δ6. *R. oryzae* and *R. miehei* lipases also react on n–6 docosapentaenoic acid (DPA; 22:5ω–6) carrying a double bond at Δ4 more weakly than on n–3 DPA (22:5ω–3) carrying a double bond at Δ7. In addition, many lipases react more weakly on DHA with the double bond at Δ4 than on EPA with the double bond at Δ5. On the contrary, *Pseudomonas* sp. lipase (Lipase AK; Amano Enzyme Co., Aichi, Japan) and *Alcaligenes* sp. lipase (Lipase QLM; Meito Sangyo Co., Aichi, Japan) do not follow this rule, and are more active on EPA than on DHA.

**FA specificity of commercially available microbial lipases**

The degree of FA specificity among lipases is different. This specificity can be classified in the following order: *C. rugosa* and *G. candidum* lipases > fungal lipases, such as *R. oryzae*, *R. miehei*, and *Thermomyces lanuginosus* lipases > bacterial lipases, such as *Pseudomonas*, *Burkholderia*, and *Alcaligenes* lipases > *C. antarctica* lipase. Immobilized *C. antarctica* lipase (Novozym 435; Novozymes, Bagsvaerd, Denmark) acts on C<sub>6</sub>–C<sub>24</sub> saturated and unsaturated FAs to a similar degree.

**Positional Specificity**

Positional specificity of lipases is also very important for oil processing. Lipases are classified into two groups: one is a 1,3-position-specific enzyme that recognizes ester bonds at the 1,3-position of TAG (recognizes primary alcohol ester), and the other is a nonspecific enzyme that recognizes all ester bonds of TAG (recognizes primary and secondary alcohol esters). Lipases from mammal pancreas and from fungi, such as *Aspergillus niger*, *R. miehei*, *R. oryzae*, *T. lanuginosus*, and *Rhizopus arrhizus*, are 1,3-position specific. Enzymes from *C. rugosa*, *G. candidum*, and *Penicillium expansum* are nonspecific and hydrolyze ester bonds at the 1,3- and 2-positions of TAG at the same velocity. Lipases from bacteria, such as *Pseudomonas*, *Burkholderia*, and *Alcaligenes* lipases, are also nonspecific, but hydrolyze ester bonds at the 1,3-position more readily than that at the 2-position. *G. candidum* produces at
least four isozymes, and the minor components, III and IV, are reported to preferentially recognize ester bonds at the 2-position of TAG. However, 2-position-specific lipase has not been found.

Positional specificity is not generally affected by reaction conditions; it does not change from 1,3-position specific to nonspecific, or from nonspecific to 1,3-position specific. However, immobilized C. antarctica lipase (Novozym 435) exceptionally changes the positional specificity. The lipase acts on the 1,3- and 2-positions of TAG at the same velocity in alcoholysis with a small amount (<1/3 molar amounts for TAG) of methanol and ethanol. Also, the lipase shows preference for the 1,3-positions in esterification of free FAs (FFAs) with glycerol, and is 1,3-position specific in alcoholysis of TAG with excess amounts (>20 molar amounts for TAG) of methanol and ethanol.

Alcohol Specificity

Zaks and Klibanov showed that lipases can act even in nearly anhydrous organic solvents, and lipases have been used widely as catalysts in chemical reactions. Alcohol specificity is also important in the field of oleo chemicals production. 1,3-Positional-specific lipases act on primary alcohols, whereas positional nonspecific lipases act on secondary alcohols including sterols and 1-menthol. Lipases also act weakly on tertiary alcohols, but not on aromatic alcohols such as tocopherols. In addition, the kind and amount of fatty alcohols change the degree of FA specificity in esterification and alcoholysis using R. oryzae, R. miehei, or C. rugosa lipase.

TAG Specificity

Lipases recognize the structure of TAGs. Tanaka and colleagues studied the activity of C. rugosa lipase on TAG with 2 mol of DHA and 1 mol of oleic acid (TAG-DDO), TAG with 1 mol of DHA and 2 mol of oleic acid (TAG-DDO), and triolein (TAG-OOO). Consequently, the velocity of hydrolysis of oleic acid ester was in the order of TAG-OOO > TAG-DDO > TAG-DDO. This specificity is called TAG specificity.

When PUFA-containing oils are hydrolyzed by taking advantage of this specificity, TAGs without PUFAs are hydrolyzed preferentially and the content of TAGs with PUFAs at the 2-position are increased efficiently. For example, hydrolysis of borage oil and AA-containing single-cell oil with C. rugosa lipase increased the content of GLA and AA at the 2-position in the resulting TAGs from 49 to 85 mol% and from 47 to 68 mol%, respectively.

Acylglycerol Specificity

The degree of hydrolysis exceeded 70% in the hydrolysis of several PUFA-containing oils with C. rugosa lipase, but the main components in the undigested acyglycerols were TAGs (>80%). This phenomenon can be explained by the fact that the lipase acts more strongly on partial acyglycerols than on TAGs. G. candidum lipase acted on monodocosahexaenoin better than on didocosahexaenoin, and is less active on tridocosahexaenoin. This specificity is called acyglycerol specificity.

Stereo and Chiral Specificities

This stereo specificity is defined as the ability of lipases to distinguish between the sn-1 and the sn-3 positions of TAG. Reports for this type of specificity are relatively recent. Examples of this type are human lingual, C. antarctica B, and dog gastric lipases for sn-3; and T. lanuginosa and Pseudomonas fluorescens lipases for sn-1 specific. Lipases are also able to differentiate between enantiomers of chiral molecules. This ability has recently become very important in producing pure chiral isomers as intermediates for drug synthesis. Many kinds of positional nonspecific lipases, such as Pseudomonas, Burkholderia, Serratia marcescens, C. rugosa, and C. antarctica lipases, have been used for the resolution of chiral compounds.

Industrial Uses of Lipase

The use of lipases for bioconversion of oils and fats has many advantages over classical chemical catalysis. Lipases operate under milder reaction conditions over a range of temperatures and pressures that minimize the formation of side products. Microbial lipases have higher potential for industrial uses because of the following: (1) a large amount of purified lipase is usually available, (2) microbial lipases are generally more stable than animal or plant lipases, and (3) microbial lipases have unique characteristics compared with plant and animal lipases. Lipases catalyze reactions in aqueous or nonaqueous systems, and in supercritical fluid.

At present, lipases are widely used as ingredients in detergents, medicines (digestive), diagnostic reagents for assay of neutral lipids and cholesterol esters in blood, catalysts for resolution of chiral compounds, food additives (hydrolysis of TAGs in food materials, production of butter, cheese, and bread flavors), and as catalysts for the production of oils and fats and related compounds. In
addition, lipases are used for the degradation of TAGs in wood in the production of paper and for the removal of fats in the production of leather. Here, the applications of lipases in the oil and fat industry are described from the viewpoint of the reactions (hydrolysis, esterification, and transesterification).

Hydrolysis

Production of FFAs

The Colgate-Emery Process operates at high temperature (250 °C) and high pressure (50 atm) for the industrial production of FFAs from tallow. Many attempts have been made to replace this chemical process with a cleaner and milder bioprocess using lipases. However, as long as the cost of lipases is not reduced dramatically, there is no sign of using lipases in the large-scale hydrolysis of tallow. Meanwhile, lipases are used for the production of unstable FFAs with hydroxyl groups and many double bonds; for example, C. rugosa lipase is used industrially for the hydrolysis of linseed and caster oils.

Production of PUFA-rich oil

Lipases generally act weakly on PUFAs; thus, the hydrolysis of PUFA-containing oils enriches PUFA in undigested acylglycerols. C. rugosa lipase is suitable for this purpose because the lipase acts most weakly on PUFAs. When tuna oil containing approximately 25% DHA was hydrolyzed with the lipase, the content of DHA in acylglycerols increased to 50% at 70% hydrolysis. Short-path distillation or n-hexane fractionation is adopted industrially to recover acylglycerols from the reaction mixture (oil layer). The content of DHA in acylglycerols obtained from a batch reaction. Several repetitions of the hydrolysis produced oils containing nearly 70% DHA. The high-DHA-containing oil has been on the market in Japan as a nutraceutical oil since 1994. Selective hydrolysis of borage oil containing GLA increased the content of GLA from 22 to 45% at 60% hydrolysis using C. rugosa lipase as a catalyst. Oil containing 57% AA was also produced by hydrolyzing a single-cell oil containing 40% AA with the same lipase. Furthermore, erucic acid can be enriched by an enzymatic process, although it is not a PUFA. A two-step enzymatic procedure for the isolation of erucic acid from rapeseed oil based on chain-length discrimination of C. rugosa lipase has been developed.

Hydrolysis of oils and fats

Starting from the middle of the 1980s, much of the laundry powder detergent sold in the United States, Japan, and Europe has contained microbial lipases, such as T. lanuginosa lipase. In waste treatment, lipases are utilized in activated sludge and other aerobic waste processes, where thin layers of fats must be continuously removed from the surface of aerated tanks to permit oxygen transport.

Dairy product flavors are produced from the hydrolysis of milk fat with calf pregastric esterases. Microbial lipases have also been used to obtain specific flavor components by the release of short- or medium-chain FAs from milk fat.

In leather manufacture, lipase is used in the processing of hides and skins to remove residual fats. It is now a common practice to utilize a mixture of lipases and proteases for this purpose.

Esterification

Production of TAGs

Conjugated linoleic acid (CLA) has various physiological functions, and is used as a nutraceutical. The primary industrial product is supplied as the FFA form, and the TAG form is produced by esterification of the CLA preparation with glycerol, using a lipase. Because this reaction is conducted in a system without the addition of water, the use of immobilized lipase is effective. When the esterification is conducted at over 50 °C, spontaneous acyl migration occurs easily. Hence, 1,3-position-specific as well as nonspecific lipases can be used. TAGs were synthesized efficiently even in an organic solvent-free system by agitating 1 mol glycerol and 3 mol FFA with immobilized C. antarctica lipase (nonspecific; 1,3-position preference) or R. miehei lipase (1,3-position specific). The degree of esterification increased by evaporating the generated water under reduced pressure with a vacuum pump, and the content of TAG reached nearly 90%. TAGs with PUFA were also synthesized using C. antarctica lipase, which acted on PUFA as strongly as on C18 FAs.

Production of DAGs

TAGs are hydrolyzed normally to 2-MAGs and FFAs by pancreatic lipase and are incorporated into intestinal mucosa. The 2-MAGs and FFAs are resynthesized to TAGs in epithelial cells, and a part of TAGs are accumulated in adipose tissue. Meanwhile, DAGs seem to be resynthesized with difficulty to TAGs after incorporation of the digested components (mainly 1(3)-MAGs and FFAs) into the intestinal mucosa, resulting in the interference of body fat increase.

DAGs are synthesized by the esterification of 1 mol glycerol and 2 mol FFAs using immobilized 1,3-position-specific lipase. In this reaction, removal of generated water increased the yield of DAGs. DAGs produced by an enzymatic process have been on the market in Japan for 'Food for Specified Health Uses' since 1999.

Production of MAGs

MAGs are one of the most used among a few (Food and Drug Administration) FDA-approved emulsifiers. MAG is
used in the food, cosmetics, and drug industries. The total world consumption is estimated at 60,000 tons annually. Currently, MAG is produced from tallow and glycerol in a continuous process using alkali catalyst at high temperature (220°C). The yield is 40–50%. Because of the high temperature, the product MAG tends to have a dark color and a burning odor. Chemical processes cannot be applied to the synthesis of MAGs with unstable FAs.

Enzymatic synthesis of MAGs has been actively studied since about 1990. Many of the reports were syntheses of MAGs with saturated and monoenoic acids by the hydrolysis of TAGs, esterification of FFAs with glycerol, glycerolysis of TAGs, and ethanolation of TAGs in organic solvent systems. An organic solvent-free system is preferable from the viewpoint of the industrial production, and several systems have been proposed; glycerolysis of TAGs with *Pseudomonas* lipase and esterification of FFAs and glycerol with lipases from *P. camembertii*, *R. oryzae*, and *C. rugosa*. An important fact can be drawn out from these reports: the yield of MAG increases in the reaction at low temperatures. This result can be explained by the melting point of MAG. Its melting point is the highest among the components (FFA, MAG, DAG, and TAG) in the reaction mixture, if the constituent FAs are the same. Because lipases act very weakly on solid-state substrates, MAG solidified at low reaction temperatures does not participate in the reaction. A high yield of MAG was therefore achieved.

These reactions take advantage of the physical property of a substrate that solidifies at low temperature. Meanwhile, activities of lipases are affected by water molecules bound to the enzymes. *P. camembertii* lipase catalyzes the conversion of MAGs to DAGs when enough water molecules bind to lipase, but does not catalyze the reaction when limited amounts of water molecules bind to the lipase. Hence, *P. camembertii* lipase produces MAGs efficiently by esterification of FFAs with glycerol even at ordinary temperature (the contents of MAGs reaches 90% at 95% esterification).

**Production of FA phytosteryl esters**

Phytosterols and their FA esters have a physiological function that reduces the level of cholesterol in blood. This function has led to the development of several ‘Foods for Specified Health Uses’ in Japan, such as salad oils with the addition of phytosterols and FA phytosteryl esters, and a margarine and a mayonnaise blended with FA phytosteryl esters.

FA phytosteryl esters are presently produced not only by the chemical esterification of phytosterols and FFAs, but also by enzymatic esterification. The solubility of phytosteryl esters with unsaturated FAs, such as linoleic and α-linolenic acids, in vegetable oil is high compared with those with saturated FAs, such as palmitic and stearic acids. Hence, phytosteryl esters with unsaturated FAs are suitable for use in salad oils. Because *C. rugosa* lipase acts on unsaturated C₁₈ FAs more strongly than on saturated C₁₆ and C₁₈ FAs, phytosteryl esters with a high content of unsaturated FAs can be produced efficiently even though the esterification is conducted with a mixture of saturated and unsaturated FAs.

**Purification of FAs**

Lipases show strong FA specificity in the esterification of FAs with lauril alcohol or l.-menthol. Hence, the reaction enriches certain FAs, in the ester fraction, and enriches the remaining FAs, in the unesterified FFA fraction. PUFAs, such as DHA, GLA, and AA, can be enriched efficiently in the FFA fraction by esterification with lauril alcohol using *R. oryzae* or *C. rugosa* lipase. In addition, *C. rugosa* lipase acted on 9cis, 11trans-CLA more strongly than on 10trans, 12cis-CLA, and esterification of a CLA mixture with lauril alcohol or l.-menthol using the lipase separated efficiently the two isomers.

**Purification of tocopherols and phytosterols**

Tocopherols, phytosterols, and FA phytosteryl esters are important useful components in vegetable oil deodorizer distillates (VODD), which is a by-product in the deodorization step of vegetable oil refining. At present, tocopherols are purified from VODD by a combination of chemical methyl esterification of FFAs, short-path distillation, methanol (ethanol) fractionation, ion exchange chromatography, and so on. Phytosterols are also purified from the by-products in the purification of tocopherols by fractionation with organic solvents, but the yield is not high. Lipases are used to improve the purification process of tocopherols and phytosterols. Lipase treatment of VODD converted phytosterols to their FA esters and FFAs to their methyl esters, and did not esterify tocopherols with FFAs. The main components after the treatment are FA methyl esters, tocopherols, and FA phytosteryl esters, which can be easily separated by short-path distillation because their boiling points differ significantly.

**Transesterification**

**Geraniol ester**

Geranyl acetate is one of the most important natural fragrances. Traditional methods such as extraction from plant materials and direct biosynthesis by fermentation are used for flavor and fragrance production. However, these methods exhibit a high cost of processing and a low yield of desired flavor component. Synthesis of geranyl acetate by lipase-catalyzed transesterification (R. meihei lipase) in hexane could reach 85% yield after 3 days of reaction.
**Marcocyclic lactones**

Marcocyclic lactones C\textsubscript{14} to C\textsubscript{16} are high-grade and expensive aromatic substances with a decidedly musky fragrance. Chemical methods of synthesis used to date have been based on the polycondensation of \(\omega\)-polyhydroxycarboxylic acids to respective polyesters and then on catalytic-thermal depolymerization and cyclization at high temperature and under vacuum. These methods are technically arduous because of the necessity to use high temperatures and high vacuum. Lipases have been used to produce macrocyclic lactones. For example: (1) lipase from Pseudomonas sp. and porcine pancreas catalyzed the lactonization of methyl esters of \(\omega\)-hydroxy acids with C\textsubscript{14}–C\textsubscript{16} producing, with high yield, monolactones and dilactones; (2) porcine pancreatic lipase lactonized various \(\gamma\)-hydroxy acid esters with high yield and high enantioselectivity; (3) lipases from C. rugosa, Pseudomonas sp. and porcine pancreas esterified dicarboxylic acids and diols with various carbon chain lengths to macrocyclic lactones; and (4) lipase from Mucor javanicus catalyzed the lactonization of 15-hydroxypentadecanoic and 16-hydroxyhexadecanoic acids to macrocyclic lactones.

**Cocoa butter substitute**

The main component of cocoa butter is TAG with palmitic and stearic acids at the 1,3-positions and oleic acid at the 2-position. Cocoa butter has a sharp melting point around body temperature, which is due to the specific structure of TAG. The fat is important as a material for making chocolate. An enzymatic process was attempted to produce a fat that has a similar melting point to that of cocoa butter. The modified fat, 1,3-stearoyl-2-oleoyl glycerol (SOS), was produced by exchange of FAs at the 1,3-positions of 2-oleoyl TAGs with stearic acid, and has been on the market since the mid-1980s. Following is a typical process: Rhizopus chinensis was grown on porous polyurethane particles. The mycelium-covered particles were collected, washed with acetone, and dried to form immobilized cells (lipase). These immobilized cells were used to produce SOS at 40% yield from olive oil and ethyl stearate by interesterification. When this process was operated at less than 100 ppm water content, the half-life of the biocatalyst (lipase) was 1.7 months.

**Human milk fat substitute**

The main component of human milk TAGs is 1,3-oleoyl-2-palmitoyl-glycerol. Gastric and pancreatic lipases in infants hydrolyze dietary fat to 2-MAGs and FFAs, and the absorption efficiency of free palmitic acid is relatively low compared with that of free unsaturated FAs. Hence, the fat absorption is higher in infants fed fats with palmitic acid at the 2-position of TAGs than the 1,3-positions. It has been hypothesized from these facts that the high absorption efficiency of human milk fat is the result of the specific positioning of palmitic acid at the 2-position of the TAG moiety.

Based on these facts, 1,3-oleoyl-2-palmitoyl glycerol was produced as a human milk fat substitute. The fat was produced by transesterification of 2-palmitoyl TAGs with oleic acid (or oleic acid ethyl ester) using immobilized 1,3-position-specific lipase. The fat substitute is presently used as an ingredient in infant formula.

**TAGs containing medium- and long-chain FAs**

TAGs in various vegetable oils, such as soybean and rape-seed oils, are mainly composed of C\textsubscript{18} long-chain FAs. As described above, TAGs are hydrolyzed to 2-MAGs and FFAs by pancreatic lipase and incorporated into intestinal mucosa. Meanwhile, ester bonds of C\textsubscript{8} and C\textsubscript{10} medium-chain FAs are absorbed and undergo rapid \(\beta\)-oxidation in the liver. Hence, TAGs containing medium-chain FAs are difficult to accumulate in adipose tissue. Attention was paid to this property of medium-chain FAs, and TAGs with long- and medium-chain FAs. They were produced industrially by interesterification of rapeseed oil and medium-chain TAG. This oil has been on the market in Japan as a 'Food for Specified Health Uses' since 2003. A reaction with powdered Alcaligenes lipase is reported to be effective for the production of this type of transesterified oil.

**Highly absorbable structured lipids**

TAGs having particular FAs at the specific position of glycerol is referred to as structured TAGs. TAGs with medium-chain FA at the 1,3-positions and long-chain FA at the 2-position (MLM-type) are hydrolyzed to 2-MAGs and FFAs faster than natural oils and fats with long-chain FAs (LLL-type), resulting in efficient absorption into the intestinal mucosa. Because PUFAs play a role in the prevention of a number of human diseases, MLM-type structured TAGs containing PUFAs are desirable as nutrition for patients with malnutrition and malabsorption of lipids, and as high-value added nutraceuticals for the elderly. Studies on the enzymatic production of structured TAGs have been conducted at many laboratories since 1995, and many processes have been proposed.

A typical production process of MLM-type structured TAGs is the same as that of the production of cocoa butter substitute and human milk fat substitute. The structured TAGs can be produced by acidolysis of natural oils with medium-chain FA or by their interesterification with medium-chain FA ethyl esters using immobilized 1,3-position-specific lipases (e.g., lipases from R. oryzae, R. miehei, and T. lanuginosa). MLM-type structured TAGs containing DHA, GLA, and AA were produced using tuna oil, borage oil, and a single-cell oil containing AA, respectively.

**Conversion of FA phytosteryl esters to free phytosterols**

Several lipases hydrolyze FA phytosteryl ester, but the reactions reached a steady state at 50% hydrolysis, owing
to the reversible reaction. The conversion of FA phytosteryl esters to free sterols by *P. aeruginosa* lipase is increased to 98% by the addition of methanol (ethanol) to the reaction mixture of FA phytosteryl esters and water. This reaction may be explained by the fact that FFAs are easily esterified with methanol to FA methyl esters and decrease the amount of FFAs in the reaction mixture to prevent the reverse reaction. Similar reaction systems convert astaxanthin FA esters to free astaxanthin efficiently.

**Biodiesel fuel**

FA methyl esters are called biodiesel fuel, which is proposed to help reduce air pollution caused by emissions from diesel engines. Biodiesel is renewable and is used as alternative diesel fuel mainly in Malaysia and Europe. The quantity of production in the world in 2006 is estimated to be 5 000 000 kl. Biodiesel fuel is presently produced from waste and surplus vegetable oils by a chemical process with an alkaline catalyst. Hence, the by-product, glycerol, contains alkali, and has to be treated as a waste material. In addition, washing of the reaction products creates waste water, including FA alkaline salts (soaps), methanol, glycerol, and alkaline catalyst. These drawbacks can be eliminated by adopting enzyme processes.

Lipases are generally unstable in short-chain alcohols, such as methanol and ethanol. A mixture of TAGs and a stoichiometric amount of methanol includes micelles of methanol because the solubility is only half a mole of TAGs. Lipases inactivate irreversibly by contact with the methanol micelles, and the inactivation disappears when conducting the reaction in a system in which TAGs and methanol are completely dissolved. Immobilized *C. antarctica* lipase converts 95–98% TAGs to their methyl esters by stepwise addition of methanol. The lipase is extremely stable in this reaction system, and can be used for more than 100 days without significant loss of activity.

EPA ethyl ester is used as a medicine for the treatment of arteriosclerosis and hyperlipemia in Japan. Although a chemical ethanolation of sardine oil is adopted in the first step for production of EPA ethyl ester, the fish oil can be converted to FA ethyl esters at more than 95% conversion by stepwise addition of ethanol.

**Enantioresolution of Esters**

One important specificity of lipases is their ability to differentiate between enantiomers of chiral molecules. Microbial lipases are increasingly used in kinetic resolution of chiral compounds that serve as synthons in the synthesis of chiral pharmaceuticals and agrochemicals. Hydroxy acids and their derivatives are major target molecules, because they constitute the framework of many chiral natural products and biologically active agents. Lipases have also been widely used for the resolution of racemic alcohols and carboxylic acids through asymmetric hydrolysis of the corresponding esters. The following are some examples of industrial and potential industrial uses of lipases in the production of enantioselective isomers.

Lipase from *C. rugosa* catalyzes the acidolysis between racemic 2-methylalkanoates and FFAs in heptane with a preference for the (S)-configured esters. The enzymatic enantioselective resolution of 2-substituted propionic acids has been the subject of intense investigation. Much of this effort has centered on the production of (R)-2-chloropropionic acid because of its high value as an intermediate in the synthesis of a number of commercially important herbicides. A substantial body of literature also exists on the production of (S)-2-arylpentanoic acids, which are valuable as anti-inflammatory agents.

Lipases are used in the synthesis of chiral synthons as intermediates for the synthesis of paclitaxel (Taxol). Taxol, a complex polycyclic diterpene, exhibits a unique mode of action on microtubule proteins that are responsible for the formation of the spindle during cell division. Taxol has been used to treat various cancers, especially ovarian cancer. Currently, Taxol is produced from the extracts of the bark of the Pacific yew tree by a cumbersome purification process. An alternative method to produce the chiral intermediate, 3R-cis-acetyloxy-4-phenyl-2-azetidinone, was carried out with lipases from *Burkholderia cepacia* and *Pseudomonas* sp. Both lipases achieve over 95% yield and 99.4% optical purity.

Lipases are used in the synthesis of a lactol, [3aS-(3aa,4a,7aa)]-hexahydro-4,7-epoxy-isobenzofuran-1-(3H)-one, which is a key chiral intermediate for the total synthesis of a new cardiovascular agent useful in the treatment of thrombolic disease. *P. fluorescens* and *B. cepacia* lipase-catalyzed reactions achieved greater than 85% yield and 97% optical purity of the chiral intermediate for the synthesis of a thromboxane A2 antagonist.

Lipases are also used in the production of an intermediate for the synthesis of an antihypertensive drug. Captopril is designated chemically as 1-[(2S)-3-mercapto-2-methylpropionyl]-1-proline. Its S-configuration is 100 times more active than its corresponding R-enantiomer. Captopril prevents the conversion of angiotension I to angiotension II by inhibiting angiotensin-converting enzyme. Lipases from both *B. cepacia* and *Pseudomonas* sp. catalyzed the production of the S-isomer at greater than 32% yield and 96% optical purity. This reaction was conducted in methanol and with immobilized lipase.

Immobilized *B. cepacia* lipase was used in an organic solvent for the selective acylation of a key alcohol intermediate, which is used for the synthesis of Camptos, a drug used in the treatment of ovarian cancer. A yield of greater than 46% conversion and 0.79 enantiomeric excess was achieved.
Lipases are also used in the production of a stereospecific isomer for the production of a β-blocker. β-Blocker is a common name for a group of antihypertensive and cardiovascular drugs that contain an arylexopropanolamine structure with an asymmetric carbon. There are over 24 drugs containing this type of moiety with over 3 billion dollars in sales annually. Traditionally, racemic arylexopropanolamine was used in the synthesis. Recently, owing to social and economic demand, it is required to use only the physiologically active pure (S)-enantiomer for the synthesis of β-blocker. Both lipases and esterases are used in this respect.

**Conclusion**

Although lipase has been perceived by scientists as being one of the most important classes of industrial enzymes, its annual sale is only about 4% of the worldwide enzyme market. The major obstacle to the practical use of lipase in the industry remains the cost of the enzymes. The significance of lipases rests on their potential rather than their current level of use. In recent years, interest in the use of enzymes as hydrolytic or synthetic chiral catalysts has risen rapidly. In particular, the search for selective enzyme inhibitors and receptors, agonists, or antagonists is one of the key factors for target-oriented research in the pharmaceutical industry. Extracellular microbial lipases are particularly suited for this application. Because of their availability, more than one dozen commercial lipases have been studied extensively by researchers in both industry and academia. Almost every pharmaceutical company has its own lipase process in producing synths for the synthesis of their patented drugs. With the rapid progress in the molecular modeling of a protein three-dimensional structure and in molecular biology, within a few years it will be possible to ‘tailor-make’ lipases with improved properties in activity, stability, and designed specificity.

**See also:** Biotransformations; Dairy Products; Enzymes, Industrial (overview); Flavor Compounds; Industrial Biotechnology, (overview); Industrial Fermentation Processes; Lipids, Production; Organic and Fatty Acid Production, Microbial

**Further Reading**


