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Lipases: Sources, Characteristics and application in Food industry

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Abstract : Lipases are used in various sectors, as pharmaceutical, food or detergency industry. Their advantage versus classical chemical catalysts is that they exhibit a better selectivity and operate in milder reaction conditions. Theses enzymes can also be used in lipophilization reactions corresponding to the grafting of a lipophilic moiety to a hydrophilic one such as sugar, amino acids and proteins, or phenolic compounds. Lipases are the most widely used class of enzymes in organic synthesis. Availability of large number of commercial preparations, their broad specificity and relatively better stability (as compared to other enzymes) in media containing organic solvents have all been contributing factors for this. This review has a sharp focus on their specificity. The recent results with catalytic promiscuity have shown that lipases are even more versatile than thought so far. These results have also prompted workers to rationalize the classification of specificity in terms of substrate promiscuity, condition promiscuity and catalytic promiscuity. The review also attempts to recast the known information on specificity of lipases in the context of enzyme promiscuity.
Key words : Lipases, Lipophilization, Application of lipases, Biotechnology.

1. Introduction

The present enzyme industry is a result of modern biotechnology boom. The world market for enzymes is expected to reach $7 billion by 2013[1]. A major fraction of the enzyme industry is represented by lipases (EC 3.1.1.3)[2]. Lipases are the most widely used class of enzymes in biotechnology[3,4]. This includes their applications in organic synthesis and kinetic resolution of racemic compounds[5,6]. There are three main reasons:

a. Foremost reason, which is often overlooked, is that industrial preparations of many lipases were available in view of their applications in early industrial enzymology. The major early application was in fat splitting. Hence, when other areas like enzyme catalysis in low water media developed, these became a convenient and preferred choice.
b. Lipases are somewhat a unique class of enzymes in carrying out reactions often in heterogeneous media. Associated with this fact is that a very large number of lipases show the phenomenon of interfacial activation. The latter phenomenon distinguishes lipases from esterases. "To withstand the denaturing effect of the interface, lipases have evolved unusually stable structures that may survive even the effect of organic solvents"[1].
c. Lipases have broad substrate specificities. Esters of fatty acids as well as alcohols of various chain lengths are hydrolyzed. Similarly triglycerides formed from long chain fatty acids of varying chain lengths are also hydrolyzed. Apart from hydrolysis, lipases can also catalyze esterification, interesterification and transesterification in low water media.

What has made lipases even more versatile is the fast developing area of catalytic promiscuity[7,8]. Despite the extensive range of microbial lipases, the use of these enzymes on an industrial scale is still limited due to their high production costs as they need to be grown in fermenters and further downstream processing and product formulation adds to cost. The technological load to implement a microbe based lipase product stands high. Further, they also have acceptability issues. This promotes the search for other sources of the enzyme[9]. Lipases have been isolated from various sources: bacterial (45%), fungal (21%), animal (18%), plant (11%), and algal (3%)[10]. While looking for advantages like low cost, specific applications, easy acceptability and their direct application as biocatalyst with partial purification, plants can be a novel source. Lipases in plant tissues mainly include non-specific lipid acylhydrolases, phospholipases A1, A2, B, C, D, monoacylglycerol, and triacylglycerol lipases. TAG[1] lipases are mainly concentrated in seeds as energy reservoirs[11,12]. They are stable in pH range of 4.0 to 9.0, temperature range of 25°C to 60°C and have varied molecular weight from 19 to 270 kDa[13]. Recently seed lipases have been the focus of attention as biocatalysts. In fact, lipases present in the crude extract from plant sources can directly catalyze hydrolysis or synthesis reactions of lipids which is one of the advantages[14]. They also have very specific applications which have been taken up in a later part of the review. However, there is a different side of the coin also. Very few plant lipases have been explored due to the complications of laborious purification steps[15].

2. Sources of lipases

Lipases are ubiquitous in nature and are found in multiple unicellular and multicellular organisms. However, yeast and fungi are one of the most important sources of lipases for industrial applications[16]. Most commercially important lipase-producing yeasts belong to the class of ascomycetous yeast, like Candida sp. Most of the lipases are extracellular and can be obtained either by submerged fermentation (SmF) or by solid-state fermentation (SSF)[17]. Lipolytic yeasts are found in a variety of oil contaminated habitats including soil contaminated with oil, wastes of vegetable oils, dairy waste and deteriorated food[18]. There are number of lipase producing yeast sources compiled by several authors, however only a few have been commercially exploited for the bulk production[19]. Some important sources are: C. antarctica, C. rugosa, Candida tropicalis, Candida curvata, Candida cylindraceae, Candida deformans, Candida parapsilosis, Candida utilis, Candida valida, Candida viswanathii, Galactomyces geotricum, Arxula adeninivorans, Saccharomyces cerevisiae, Yarrowia lipolytica, Trichosporon fermantans, Trichosporon asahii, Rhodotorula mucilaginosa, and Aureobasidium pullulans[9].

3. Structure of lipases

The three-dimensional structures of many lipases have been determined by X-ray crystallography. Table 1 shows the illustrative list of lipases whose X-ray structures have been determined. Based upon these studies, the following structural features common to all lipases can be identified: (1) All the lipases are members of "α/β-hydrolase fold" family i.e., these have a structure which is composed of a core of predominantly parallel β strands surrounded by α helices[20]. (2) The active nucleophilic serine residue rests at a hairpin turn between a β strand and an α helix in a highly conserved pentapeptide sequence Gly-X-Ser-X-Gly, forming a characteristic β-turn-α motif named the 'nucleophilic elbow'[20]. Lipase B from Candida antarctica (CALB) does not have a conserved pentapeptide sequence Gly-X-Ser-X-Gly, around the active site which is present in most of the other lipases[21]. (3) The active site of lipases is formed by a catalytic triad consisting of amino acids serine, histidine and aspartic acid/glutamic acid[20]. The active site of both lipases and proteases are chemically similar but structurally different[22].

The seryl hydroxyl group in lipases is oriented differently than in serine proteases and this result in inverted stereochemistry of the catalytic triad[23]. (4) Presence of a lid or flap composed of an amphiphilic α helix peptide sequence that covers the active site[7]. In case of lipase from Geobacillus thermocatenulatus, lid has a complex structure involving a large percentage of the amino acids of the enzyme and forms a double lid[20]. Lipase B from C. antarctica has a very small and simple lid which does not fully isolate the active center of the enzyme in the closed form[21]. Lipase from guinea-pig has a "mini-lid" which is composed of only five amino acids[24]. (5) These have four substrate binding pockets for triglycerides: an oxyanion hole and three pockets

[1] Abbreviations used: TAG, triacylglycerol acylhydrolase; HMFS, human milk fat substitutes; CPL, Carica papaya lipase; sn-BSP, 1-butyroyl-2-stearoyl-3-palmitoyl-snglycerol; TG, triacylglycerol; GA3, gibberellic acid; ABA, abscisic acid; BR, brassinosteroids; PMSF, phenyl methane sulfonyl fluoride; FA, fatty acid.

accommodating the fatty acids bound at positions sn-1, sn-2, and sn-3. Two backbone amides of a residue in the N-terminal region of the lipase and the C terminal neighbor of the catalytic serine form the oxyanion hole[25].

Table 1- Illustrative list of lipases whose X-ray structures have been determined along with their specificity (www.pdb.org).

Lipase source		Specificity	References
Bacterial	Bacillus thermocatenulatus	1,3-Regiospecific	[26]
	Burkholderia glumae (Pseudomonas glumae)	Non-specific	[3]
	Burkholderia cepacia (Pseudomonas cepacia)	Non-specific	[3]
	Bacillus subtilis	-	-
	Chromobacterium viscosum	Non-specific	[27]
	Pseudomonas fluorescens[a]	Non-specific	[27]
Fungal	Aspergillus nigera	1,3-Regiospecific	[27]
	Candida rugosa (Candida cylindracea)	Non-specific	
	Candida antarctica A[b]	Trans specific	[26]
	Candida antarctica B	1,3-Regiospecific	[3]
	Geotrichum candidum	cis-▲^{9}(unsaturated fatty acids)	[28]
	Mucor javanicus[a]	1,3-Regiospecific	[3]
	Penicillium camembertii (Penicillium cyclopeum)	1,3-Regiospecific	-
	Penicillium expansum	-	[27]
	Rhizomucor miehei (Mucor miehei)	1,3-Regiospecific	[28]
	Rhizopus delemar	1,3-Regiospecific	[3]
	Rhizopus oryzae	1,3-Regiospecific	[4]
	Rhizopus niveus	1,3-Regiospecific	[29, 4]
	Thermomyces lanuginosa (Humicola lanuginosa)	1,3-Regiospecific	[5]
	Yarrowia lipolytica	1,3-Regiospecific	
Plant	Brassica napus (rapeseed) [a]	1,3-Regiospecific	
Animal	Canis lupus familiaris (dogs)	-	-
	Equus caballus (equine)	-	-
	Porcine pancreatic lipase[a]	1,3-Regiospecific	[3]
	Human pancreatic lipase	1,3-Regiospecific	[3]
	Sus scrofa (wild bear)	-	-

4. Mechanism of lipase action

Due to the similarity of the catalytic triad found in lipases and proteases, the mechanism of lipase catalysis is similar to that of serine protease catalysis i.e., involves formation of two tetrahedral intermediates[30]. The mechanism involves the nucleophilic attack of hydroxyl group of serine residue (present in the active site) on carbon from the ester bond of susceptible substrate. This results in the formation of tetrahedral intermediate which then loses an alcohol molecule to give an acyl-enzyme intermediate. A water molecule then attacks the complex (nucleophilic attack) to give tetrahedral intermediate, which finally, loses an acid molecule to give the enzyme in its native form[4].

5. Food industry

The advantage of using plant lipases in food industries as compared to other sources of lipase is their acceptability in comparison to microbial lipases. Plant lipases have improved stability in solvent catalyzed reactions such as interesterification. Additional importance of plant lipases are in their low cost of production and downstream processing. In this context, Carica papya lipase (CPL) has been used successfully by Mangos et al. in the synthesis of low-calorie short and long-chain triacylglycerols (TAG) for use in infant formulae.

Papaya lipase has also been used to produce structured triacylglycerols using interesterification reactions of ethyl esters with tripalmitin[31]. The unavailability and high cost of human milk fat can be compromised through the synthesis of human-resembling milk fat by carrying out transesterification of tripalmitin with fatty acids of rapeseed oil using papaya latex[32]. Recently, human milk fat substitute has been synthesized using CPL self-immobilized in papaya latex as a biocatalyst and used as a low-cost alternative to commercial lipases[33]. Other examples are in the use of CPL catalyzed interesterification of palm oil, which is a low cost fat, for the synthesis of cocoa butter equivalent which can be used in the production of chocolates at a much cheaper rate. This also produces fewer by-products as compared to chemical synthesis[34]. On similar lines, fat and oil modification using CPL based on sn-3 stereoselectivity in TAG interesterification has also been reported by Villeneuve et al[35]. From the above, it can be concluded that CPL possess various useful commercial application and is an inexpensive plant enzyme preparation from the crude papaya latex[37].

6. Plant lipase purification and recombinant production – challenges and scope Source

Lipases have been purified from various plant parts. It has been reported from latex of C. papaya and scutella of Z. mays. Oilseed lipases have been reported to be localized in oil bodies[37]or glyoxysomes[38]. Table 2 summarizes some of the plant lipases with their corresponding plant parts used for extraction. Many plant parts such as leaves of Triticum L. species[39], whole plant parts of Ricinus communis[40], oat bran, etc., are rich in phenolic content.

Table 2- Sources of plant lipases.

Plants	Family	Plant part	References
Coconut (Cocos nucifera linn)	Arecaceae	Seed	[41]
Sunflower (Heliantus annuus L.)	Arecaceae	Seed	[42]
Vernonia galamensis	Arecaceae	Seed	[43]
Canola (Brassica napus L.)	Brassicaceae	Seed	[44]
Jacaranda mimosifolia	Brassicaceae	Nectar	[45]
Bobacco fruit (Carica pentagona)	Caricaceae	Latex	[46]
Papaya (Carica papaya)	Caricaceae	Latex	[47]
Dianthus caryophyllus	Caricaceae	Petal	[48]
Castor beans	Caryophyllaceae	Seed (endosperm)	[49]
Euphorbia characias	Euphorbiaceae	Latex	[50]
Euphorbia wulfenii	Euphorbiaceae	Latex	[4]
French Peanut (Panchira aquatic Bombacaceae)	Euphorbiaceae	Seed	[51]
Lupin (Lupinus luteus L.)	Fabaceae	Seed	[52]
Laurel (Laurus nobilis L.)	Fabaceae	Seed	[53]
Bean (Pentaclethra macrophylla Benth L.)	Leguminosae	Seed	[54]
Linseed (Linum usitatissimum L.)	Linaceae	Seed	[55]
Ficus carica	Moraceae	Latex	[56]
Sesame (Sesamum indicum L.)	Pedaliaceae	Seed	[57]
Barley (Hordeum vulgare L.)	Poaceae	-	[58]
Oat (Avena fatua)	Poaceae	Seed	[59]
Rice (Oryza sativa)	Poaceae	Seed	[60]
Oryza sativa cv. Dongjin	Poaceae	Seed coat	[61]
Oryza sativa L. ssp. Indica var. IR64	Poaceae	Seed	[62]
Sorghum (Sorghum bicolor L.)	Poaceae	Seed	[63]
Wheat Lipases (Triticum aestivum L.)	Poaceae	Seed	[64]
Black-Cumin (Nigella sativa L.)	Ranunculales	Seed	[65]
Almond (Amygdalus communis L.)	Rosaceae	Seed	[66]
Lycopersicon esculentum	Solanaceae	SeedS	[67]

7. Applications of lipases

As mentioned earlier, lipases can carry out not only hydrolytic reactions but also synthetic reactions like esterification, acidolysis, alcoholysis etc. These can carry out reactions in both aqueous and organic media, have broad substrate specificity, and can catalyze variety of chemo-, regio- and enantioselective biotransformations[5,6]. This versatility of lipases makes them the enzymes of choice for application in food, detergent, pharmaceutical, leather, textile, cosmetic and paper industries[5]. One of the most important reasons for the large number of applications of lipases is that they exhibit regio, substrate and stereospecificity. As mentioned earlier some lipases Exhibit 1,3 regiospecificity and can be used to (inter)esterify natural triglyceride in a regioselective fashion. For example, cocoa butter equivalents [predominant triglycerides of cocoa butter are glycerols with oleic acid (O) in the sn-2-position and stearic (S) and palmitic (P) acids in the sn-1- and sn-3-positions (i.e., SOS and SOP)] have been produced by lipase-catalyzed interesterification of suitable natural triglycerides, such as middle fraction of palm oil (containing palmitic acid moiety at 1 and 3 positions and oleic acid at 2, POP) or sunflower oil (having a high content of oleic acid, OOO) with stearic acid or tristearin (SSS)[68]. Lipases discriminate against omega 3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and hence lipase catalyzed hydrolysis has been utilized for the production of omega 3 PUFA concentrates[69]. Kahveci and Xu[70] carried out the enrichment of omega 3 polyunsaturated fatty acids (PUFA) in the glyceride fraction of salmon oil by C. rugosa lipase (CRL)-catalyzed hydrolysis. Table 3 shows some of the industrial applications of lipases.

Table3- Some of the industrial applications of lipases.

Lipase reaction	Industry	Product/application	References
Hydrolysis of fats	Detergent	Removal of oil stain from fabrics	[5]
Hydrolysis of milk fat, cheese ripening.	Dairy	Development of flavoring agents in milk, cheese, and butter	[71]
Hydrolysis	Bakery, brewery and food	Improvement of flavor and quality in beverages, meat, fish products	[72]
Hydrolysis	Leather	Leather products	[5]
Hydrolysis	Paper	Paper with improved quality	[72]
Transesterification oils/fats	Natural oils	Cocoa butter	[68]
Esterification and transesterification	Flavor and fragrance	Synthesis of natural flavor esters	[73]
Glycerolysis of fats/oils	Surfactants	Monoglycerides for surfactants	[3]
Resolution of racemic alcohols/esters	Drugs and pharmaceuticals	Building blocks for chiral drugs and insecticides	[5]
Transesterification	Fuel	Biodiesel	[74]
Acylation of sugar alcohols	Surfactants	Sugar monoacyl esters for surfactants	[75]

8. Conclusion

Lipases are versatile enzymes that can be used for various kinds of biocatalyzed reactions. Owing to their regioselectivity, their mild reactions conditions, they can be often considered as more interesting than classical chemical catalysts. Besides their application in oils and fats processes, these enzymes have proved to be very attractive for others lipase-catalyzed reactions with non-natural substrates. In particular they appear to be very effective for the synthesis of molecules involving the grafting of a lipophilic moiety or a hydrophilic one. In such reactions, various parameters and strategies can be modulated in order to improve reaction yields and kinetics. Among these parameters, appropriate choice of reaction medium, control of water activity and water content of the systems, nature of acyl donor, subtract ratio appear to be the keys for optimized reaction rates and conversion yields. Recently, some new applications of lipases have been described in the field of the modifications of natural compounds such as phenolic acids or polyphenols. These biocatalyzed reactions intend

to modify the hydrophilic/lipophilic properties of the initial molecules to obtain new products with multi-functional properties combining for example, microbial, antioxidant and emulsifying properties. Although the literature on such lipase-catalyzed reactions is still scarce, it is expected to be further extended and give access to new products and bioactive molecules. Of course, a lot of work and studies are still to be done for a potential industrial application of such lipase-catalyzed reactions. The gap between the feasibility studies on the lab scale and the corresponding industrial development is very large. Notably, improvements in reactions yields or enzyme performances must be carried out. However, it appears evident that lipase applications will be more and more extended allowing the synthesis of very specific compounds with added values in various fields of food, pharmacy or cosmetic industry.

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