Cold-adapted bacterial lipolytic enzymes and their applications

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Keywords: lipolytic enzymes; cold-adapted; industrial application

Abstract: Lipolytic enzymes, esterases and lipases catalyze the hydrolysis and transesterification of fatty acid esters. This class of enzymes is the most important group of biocatalysts for biotechnological applications. From this perspective, cold-active lipolytic enzymes from cold adapted bacteria display attractive properties for some industrial purposes, including high catalytic activity at low temperatures and low thermostability. To date, such enzymes were isolated from *Pseudoalteromonas* sp. 643A, *Pseudomonas* sp. B11-1, *Psychrobacter* sp. C18, *Pseudoalteromonas haloplanktis* TAC125,*Pseudomonas mandelii, Sorangium cellulosum*So157-2,*Psychrobacter* sp. Ant300, *Streptomyces coelicolor* A3(2), *Pseudoalteromonas arctic, Pseudomonas* sp. 7323 and characterized. In summary, cold-active lipolytic enzymes are promising biocatalysts for organic synthesis and as enzymatic additives in cleaning agents.

1. Introduction

Lipolytic enzymes, both esterases and lipases,belong to the enzyme class of hydrolases. They catalyze hydrolysis of triacyloglycerols to diacylglycerol, monoacylglycerol, fatty acids and glycerol. Esterases hydrolyze solutions of water-soluble short acyl chain esters (chain length of <10 carbon atoms). In contrast to that, lipases are active against water-insoluble long chain triacylglycerols (chain length of >10 carbon atoms).Moreover, lipolytic enzymes have the ability to catalyze esterification, interesterification, alcoholysis, acidolysis and aminolysis. Some properties make these enzymes one of the most interesting biocatalysts. Some of them exhibit stability under the harsh conditions, regioselectivity, stereoselectivity or chemoselectivity of catalyzed reactions, respectively. Furthermore, lipolytic enzymes possess broad substrate specificity (Arpigdny and Jaeger, 1999).

To date, lipolytic enzymes have found extensive applications in the leather, food, detergent, pharmaceutical, textile, cosmetic and paper industries.

In the detergent industry, enzymes are widely used to remove clothing stains from various types of fabric. Lipases are capable of removing fat-containing stains includ-

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ing butter, fat based sauces, soups, fried fat, salad oils, human sebum and lipstick. Lipolytic enzymes used in detergents are active and stable in alkaline environments (pH between 10 and 11) and in temperatures ranging from 30°C to 60°C. Moreover, these lipases are active in the presence of surfactants and other enzymes like proteases. Such lipolytic enzymes have been isolated from *Pseudomonas mendocina, Pseudomonas alcaligenes, Pseudomonas glumae, Bacillus pumilis* and used in the manufacture of detergents (Hasan et al., 2006; Gupta et al., 2004)

Lipolytic enzymes are also used to modify properties of fats or oils to make them more useful in food industries. These modifications include changes in the position of the fatty acid on the glycerol backbone or replacing fatty acid residues by others. Cocoa butter is a mixture of oil and fat composed of triglycerides. Major components are stearic acid, palimitic acid and oleic acid. The high price of cocoa butter has encouraged the search for alternatives. Today, immobilized lipase from *Rhizomicor miehei* is used to produce a cheaper cocoa butter substitute. Moreover, lipases are used for flavour enhancement and improving the aroma of foods. Lipolytic enzymes can improve bread quality. Lipases from *Aspergillus niger, Rhizopus oryzae and Candida cylindracea*are used in bakery industry (Hasan et al., 2006). Lipase from *Rhizomucor meihei* is also used as a biocatalyst to produce isopropyl myristate, isopropyl palmitate and 2-ethylhexylpalmitate. These compounds are used as an emollient in personal care products such as skin and sun-tan creams, bath oils etc.

Lipolytic enzymes are used frequently as catalysts in the synthesis of fragrance compounds. The most prominent one is (-)-menthol. Lipase from *Burkholderia cepacie* is used to isolate enantiomerically pure (-)-menthol. Another example is the synthesis of (-)-menthyl jasmonate by commercially available lipase LipP (Jaeger and Eggert, 2002).

It is important to note that lipolytic enzymes can catalyze asymmetric synthesis. Therefore, both lipases and esterases are considered to be the most useful enzymes for regioselective esterification or transesterification of polyfunctional compounds. They can be used in kinetic resolution of racemic alcohols, acids, esters, amines and desymmetrization of prochiral compounds (Gotor-Fernandez and Gotor, 2007). For instance, the lipase from *Serratia marcescens*is used in the pharmaceutical industry for the production of drugs such as Diltiazem. Diltiazem is a calcium channel blocker (Bornscheuer and Kazlauskas, 1999). Moreover, lipolytic enzyme from *Serratia marcesces* is useful to produce ketoprofen, a non-steroidal anti-inflammatory drug. Lipase from *Pseudomonas cepacia* is capable of synthesizing HIV-protease inhibitors. Lipase CAL-B catalyzes desymmetrization of the prochiral compound diethyl 3-(3',4'-dichlorophenyl)-glutarate, an intermediate in the synthesis of neurokinin receptor antagonists (Sangeetha et al., 2011).

Enzymatic desizing of materials in the fabric has advantages over the traditional process,which uses acid or oxidizing agents. For this purpose, bacterial lipases from *Pseudomonas cepacia, Pseudomonas fragi, Pseudomonas fluorescens, Pseudomonas stutzeri* can be used (Hasan et al., 2006). Lipolytic enzymes are also used in the leather industry. These enzymes are employed in bating, soaking and degreasing stages (Sangeetha et al., 2011).

In summary, both esterases and lipases are biocatalysts useful in different industrial applications. Recently, much attention has been to the application of cold-active esterases and lipases. What is particularly important is that the use of cold-active

enzymes in industrial applications offers possible economic benefits through energy saving.

2. Cold-active lipolytic enzymes

A variety of microbial cold-adapted lipases and esterases with different enzymatic properties and substrate specificities have been found (Tab. 1).

Tab. 1. Properties of bacterial cold-adapted lipolytic enzymes.

Name	**Source**	**MV kDa**	Optimum		Prefered substrate	Family	References
			pH	**Temp °C**			
CH2A	metagenomic DNA	34.7	11.0	20	*p*-NP-propionate	α/β hydrolase	(Hu et al., 2012)
EstIM1	metagenomic DNA	34.0	8.0	40	*p*-NP-propionate	IV(HSL)	(Ko et al., 2012)
EstK	*Pseudomonas mandelii*	33.0	8.5	40	*p*-NP-acetate	α/β hydrolase	(Hong et al., 2012)
EstA	*Pseudoalteromona* ssp. 643A	23.1	8.0	35	*p*-NP-acetate	II(GDSL)	(Cieśliński et al., 2007)
EstO	*Pseudoalteromonas arctica*	44.1	7.5	25	*p*-NP-butyrate	α/β hydrolase	(Khudar et al., 2010)
LipP	*Pseudomonas* sp.B11-1	33.7	8.0	45	*p*-NP-butyrate	α/β hydrolase	(Choo et al., 1998)
PsEstI	*Pseudomonas* sp.B11-1	69.0	8.0	45	*p*-NP-butyrate	α/β hydrolase	(Suzuki et al., 2003)
LipA	*Pseudomonas* sp.7323	64.5	9.0	30	*p*-NP-capyrate	III	(Zhang and Zeng, 2008)
LipX	*Psychrobacter* sp.C18	35.0	8.0	30	*p*-NP-myrirate	α/β hydrolase	(Chen et al., 2011)
LipA	*Sorangium cellulosum* So157-2	35.6	8.0	30	*p*-NP-butyrate	α/β hydrolase	(Chen et al., 2011)
EstC	*Streptomyces coelicolor*A3(2)	34.7	8.5-9.0	35	*p*-NP-butyrate	V	(Brault et al., 2012)
PsyEst	*Psychrobacter* sp. Ant300	43.7	no data	35	*p*-NP-hexanote	IV(HSL)	(Kulakova et al., 2008)
Lip1	*Pseudoalteromonas haloplanktis* TAC125	no data	8.5	40	*p*-NP-capyrate	α/β hydrolase	(Pascale et al., 2008)

Gene encoding esterase CHA2 was isolated from Antarctic mineral soil metagenomic library. The gene was found to have an open reading frame of 1044 base pairs. The molecular weight of the esterase CHA2 was determined to be 34.7 kDa and an isoelectric point to be 9.21. In *E. coli* the esterase was produced as inclusion bodies. The enzyme refolding from inclusion bodies was successful and it was thus possible to determine the enzyme activities. The enzyme exhibited activity in temperature range of 5 to 40°C,with the maximum activity at 20°C. Esterase CHA2 was stable between pH 5 and 12, with the optimal pH reported around pH 11. The enzyme showed maximum activity for esters of short-chain (C3) fatty acids (Hu et al., 2012). The sequence of *cha*2 gene was deposited with GenBank under the accession number EU874394.

The gene *est*IM1 encoding novel esterase was isolated from a soil metagenomic library. The soil sample was collected from the Ibam mountain in Republic of Korea. The DNA sequence of *est*IM1 has been submitted to EMBL under accession number HM802891. Esterase EstIM1 has a molecular weight of 34 kDa and an isoelectric point of 4.32. Testing of an effect of temperature on the enzyme activity showed that EstIM1 was stable between 1 and 50°C. The enzyme was active in range of pH, from 6 to 9 (Ko et al., 2012).

A novel esterase EstO from *Pseudoalteromonas arctica* has a molecular mass of 44.1 kDa. The enzyme showed maximum activity at 25°C and pH 7.5. EstO displayed the highest activity towards butyrate *p*-nitrophenyl (C4). The sequence of the gene *est*O was deposited in the EMBL database under accession number FN666254 (Khudar et al., 2010).

Lipolytic enzyme EstK from *Pseudomonas mandelii* has a molecular mass of 33 kDa. The highest activity among the *p*-NP esters examined was measured by the utilization of C2 ester *p*-NP acetate. Esterase EstK was stable at temperatures between 4 and 40°C. The temperature optimum was calculated to be 40°C. The esterase was active in pH 8.5. The DNA sequence of *estK* was deposited with EMBL under the accession number AEW10549.2 (Hong et al., 2012).

Lipolytic enzyme EstA (PDB code 3hp4) has a molecular weight of 23.0 kDa. The sequence of EstA consists of 207 amino acid residues. The enzyme was stable between pH 9 and 11.5. The optimal activity occurred when the pH level was 8.0 at a temperature of 35°C. In *E. coli* this esterase was produced as inclusion bodies (Cieśliński et al., 2007).

The DNA sequence of the gene encoding esterase PsEstI was deposited with EMBL under the accession number BAC21259.1. This enzyme from *Pseudomonas* sp. B11-1, exhibited the highest esterase activity with *p*- nitrophenyl butyrate.The enzyme was active in pH ranging from 2.2 to 11.9, with the optimum pH of 8.0 and the optimum temperature of 45°C.This enzyme was expressed in *E. coli* as inclusion bodies. The esterase was successfully refolded (Suzuki et al., 2003).

The lipolytic enzyme from *Pseudomonas* sp. 7323(GenBank: AM229328.1) has a molecular mass of 64.5kDa. This protein consist of 617 amino acid residues. It had substrate specificity towards esters of short- and medium- chain fatty acids. LipA was stable over a wide pH range (between pH 6 and 12).The optimum activity was recorded at pH 9.0 and the temperature of 30°C (Zhang and Zeng, 2008).

EstC from *Streptomyces coelicolor* A3(2) has a molecular weight of 34.7kDa. The enzyme displayed the optimum pH of 8.5-9.0 and the optimum temperature of 35°C.

EstC has substrate preference for *p*-nitrophenyl butyrate (Brault et al., 2012).

The nucleotide sequence of lipA from *Sorangiumcellulosum* So0157-2(GenBank: JF739860.1) encodes a lipolytic enzyme with a predicted molecular mass of 35.6 kDa. The enzyme consist of 326 amino acid residues. The optimal pH was indicated to be pH 8.0. LipA exhibited maximum activity at 30°C (Chen et al., 2011).

LipP from *Pseudomonas* sp. B11-1 has molecular weight of 33.7 kDa. The enzyme consists of 308 amino acid residues. The highest activity among the *p*-NP esters examined was measured by the utilization of the C4 ester *p*-NP butyrate. The optimal activity occurred when the pH level was 8.0 at a temperature of 45°C (Choo et al., 1998).

Lipase isolated from *Psychrobacter* sp. C18 consists of 315 amino acid residues. The molecular weight of LipX was determinate to be 35.0 kDa. It had substrate specificity towards esters of long chain fatty acids, particularly, *p*-nitrophenyl myristate. LipX had the optimum activity at 30°C and pH 8.0. The enzyme was stable over a wide pH range (between pH 6.0 and 11.0). The DNA sequence of the gene *lipX* was deposited with EMBL under accession number ADD74206.1 (Chen et al., 2011).

The lipolytic enzyme from *Psychrobacter* sp. Ant300 exhibited the highest activity with *p*-nitrophenyl hexanoate. This esterase has a molecular mass of 43.7kDa. PsyEst displayed an optimum temperature of 35°C. The nucleotide sequence of the gene encoding esterase was deposited with EMBL under accession number BAD06009.1 (Kulakova et al., 2008).

Lip1 from *Pseudoalteromonas haloplanktis* TAC125 consist of 456 amino acid residues (NCBI: YP_338604.1). The enzyme exhibits optimal activity around 45°C, at pH 8.5. Lip1 had substrate specificity towards esters of long-chain fatty acids (C10) (Pascale et al., 2008).

3. Conclusions

The knowledge of bacterial lipases and esterases has increased significantly in the last decade. Every year, many novel lipolytic enzymes are being discovered and characterized. Many of them have been successfully utilized in industrial processes. In this light, cold-adapted enzymes are promising to replace conventional enzyme processes because of their high catalytic activity at low temperatures and low thermostability.

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