Biochemical and Biophysical Research Communications 424 (2012) 285–289

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Molecular basis for substrate selectivity of a mono- and diacylglycerol lipase from *Malassezia globosa*

Lu Liu [a], Chongliang Gao [a], Dongming Lan [b], Bo Yang [a,*], Yonghua Wang [b,*]

[a] School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, China
[b] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China

ARTICLE INFO

Article history:
Received 13 June 2012
Available online 27 June 2012

Keywords:
SMG1
Open conformation
Mono- and diacylglycerol lipase
Substrate selectivity

ABSTRACT

The lipase from *Malassezia globosa* (SMG1) was identified to be strictly specific for mono- and diacylglycerol but not triacylglycerol. The crystal structures of SMG1 were solved in the closed conformation, but they failed to provide direct evidence of factors responsible for this unique selectivity. To address this problem, we constructed a structure in the open, active conformation and modeled a diacylglycerol analogue into the active site. Molecular dynamics simulations were performed on this enzyme-analogue complex to relax steric clashes. This bound diacylglycerol analogue unambiguously identified the position of two pockets which accommodated two alkyl chains of substrate. The structure of SMG1-analogue complex revealed that Leu103 and Phe278 divided the catalytic pocket into two separated moieties, an exposed groove and a narrow tunnel. Analysis of the binding model suggested that the unique selectivity of this lipase mainly resulted from the shape and size of this narrow tunnel, in which there was no space for the settlement of the third chain of triacylglycerol. These results expand our understanding on the mechanism underlying substrate selectivity of enzyme, and could pave the way for site-directed mutagenesis experiments to improve the enzyme for application.

1. Introduction

Lipases are triacylglycerol hydrolases (EC 3.1.1.3) that catalyze the hydrolysis of long chain, insoluble triacylglycerol to glycerol and fatty acid. Although lipases from diverse sources, ranging from microorganisms to mammalian, conform to the α/β hydrolase fold in structure, they are, in general, highly variable in size and share low identity in sequence [1]. Unsurprisingly, lipases possess wide substrate selectivity.

A subfamily, termed as partial glycerol lipases (PGLs), which are considerably different from typical lipase, displays a unique selectivity, strictly specific for mono- and/or diacylglycerol but not triacylglycerol. Sequence analysis suggests that PGLs share relatively low homology with other known triacylglycerol lipases. Several lipases from fungi and mammalian were experimentally identified as PGLs [2–5], and found that they play importantly physiological roles in animals, from complete hydrolysis of monoglycerides in the adipocyte to regulation of the transmission of 2-arachidonoylglycerol in brain [3,5].

A secreted lipase isolated from *Malassezia globosa* (SMG1) was observed that it was active against diolein, but not triolein [6–8]. Recently, the crystal structures of SMG1 (PDB ID: 3UUE and 3UUF) were determined in closed conformation, and revealed that the catalytic triad (Ser171–Asp228–His281) of SMG1 was shielded from the solvent by a surface loop [9]. By itself, a structure in closed conformation can tell little information on this unique selectivity. Therefore, a structure in the open, active conformation is required.

At present, few experimental and computational studies were carried out to explore the mechanism underlying the unique selectivity of mono- and diacylglycerol lipases: no crystal structure in the open conformation was reported, and only one structural model was proposed for explaining the selectivity of a mono- and diacylglcyerol lipase from *Penicilliurn camembertii* (PCL), in which the distance between the carboxyl carbon in substrates and the nucleophilic O_γ of Ser145 was related to the hydrolysis activity [10,11]. Apparently, this model used to account for selectivity is oversimplified.

In this work, in order to investigate the molecular basis responsible for this unique selectivity, we constructed a structural model in the open, active conformation and modeled a substrate analogue to the active site to mimic the interactions between enzyme and substrate. Molecular dynamics simulations (MD) were performed to relax steric clashes in the SMG1-analogue complex and to identify the binding sites. The model of the SMG1-analogue complex revealed that Leu103 and Phe278 divided the whole catalytic pocket into two separated moieties, an exposed groove and a narrow tunnel, which were restricted to accommodating the two alkyl

* Corresponding authors. Fax: +86 020 87113842.
E-mail addresses: yangbo@scut.edu.cn (B. Yang), yonghw@scut.edu.cn (Y. Wang).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.108

chains of diacylglycerol only. The present work described the open structure of SMG1 in complex with diacylglycerol analogue, which allowed a detailed discussion of the structurally important parts responsible for the unique selectivity of this lipase.

2. Methods and materials

2.1. Construction of the structure in the open conformation

The crystal structures of SMG1 were solved in the closed conformation and the lid domain showed as a loop. However, according to JPred, this region was predicted as a loop containing α-helix, well conserved in the open conformation of lipases [12]. Moreover, during the opening of lid, similar conformational changes have been observed in *Geobacillus thermocatenulatus* lipase and *Pseudomonas cepacia* lipase [13,14]. The loop of the lid domain in these lipases partly reorganizes into the α-helix. Therefore, we assume the lid of SMG1 adopting α-helix form in the open conformation.

The structure of SMG1 in the open conformation was constructed by homology modeling based on the crystal structure of *Rhizomucor miehei* lipase (RML) in complex with the diethyl p-nitrophenyl phosphonate (PDB ID: 4TGL) by the MODELLER package [15,16]. Then we substituted the coordinates of the lid region of the homology model (Thr101–Trp116) for the identical part in the crystal structure, and integrated it with the other segments in the crystal structure. Therefore, the constructed structure and the crystal structure were identical except for the lid region.

2.2. Construction of a model in complex with substrate analogue

In order to provide insight into the substrate selectivity mechanism of SMG1, we modeled the phosphonate from *pseudomonas aeruginosa* lipase (PDB ID: 1EX9) into the active site [17]. This phosphonate is a triacylglycerol analogue, so one fatty acid chain at the *sn-1* or *sn-2* should be deleted for mimicking a diacylglycerol bound to SMG1. In this work, the *sn-1* moiety was removed randomly. MD simulations were carried out to fully relax the steric clashes occurred in this enzyme-substrate analogue complex.

2.3. Molecular dynamics (MD) simulations

The MD simulations were performed by the DISCOVERY STUDIO package version 3.1 (Accelerys Inc.) on an IBM Windows workstation to relax the steric clashes between SMG1 and substrate analogue.

The SMG1-analogue complex was first solved in an orthorhombic box with 8249 water molecules. And prior to MD simulation, this complex system was subject to twice energy minimizations. In the first minimization, the steepest descent algorithm was employed with the atoms of SMG1-analogue complex constrained, until the final RMS gradient was less than 0.1 kcal/(mol × Å). In the next minimization, all atoms were allowed to move, and the conjugate gradient algorithm was used to make the final RMS gradient converged to 0.0001 kcal/(mol × Å).

The system was heated from 50 to 300 K in 4 ps and then equilibrated at 300 K for another 4 ps. A subsequent production was performed at a constant temperature of 300 K and a constant pressure of 1 atm, giving a total simulation of 30 ps to fully relax the whole system.

The CHARMM force field was employed during all the MD simulations. Periodic boundary condition was used for the simulation box at three dimensions. A cut-off of 14 Å was used for counting the Van der Waals interactions whereas the Particle Mesh Ewald (PME) algorithm was applied to compute the long range electrostatic interactions. An integration step of 2 fs was used for the MD simulations.

3. Results and discussions

3.1. Assessment of the constructed structure

PGLs are strictly specific for mono- and/or diacylglycerol, but not triacylglycerol, considerably differing from triacylglycerol lipases. The molecular mechanism for this unique substrate selectivity is still not clear. SMG1 is one of PGLs and its crystal structures were solved in the closed conformation. However, these structures failed to provide direct evidence for explaining its substrate selectivity. Therefore, we constructed the open structure of the SMG1 in complex with a substrate analogue to explore the interactions between enzyme and substrate.

Extensive experimental and computational studies show that upon the opening of the lid, the functional consequences of conformational change are similar: (1) the buried active site in the closed conformation is accessible in the open state; (2) the hydrophobic residues lining the catalytic pocket are exposed to solvent; (3) the oxyanion hole is formed to stabilize the tetrahedral intermediates [16–21]. Our constructed structure of SMG1 satisfied all of these.

In the constructed structure of SMG1, the lid domain adopted a quite different conformation containing an amphiphilic α-helix and the movement of lid uncovered the catalytic triad (Fig. 1). Leu106, Leu110 and Ala113 located in this amphiphilic helix together with the residues (Try56, Phe138, Leu172, Pro201, Val230, Phe278, Ala293, Met294) lining the catalytic pocket, were exposed to solvent. Additionally, in the crystal structure, the backbone nitrogen atom of Thr101 was not conveniently oriented toward the catalytic center, while in the constructed structure, it was

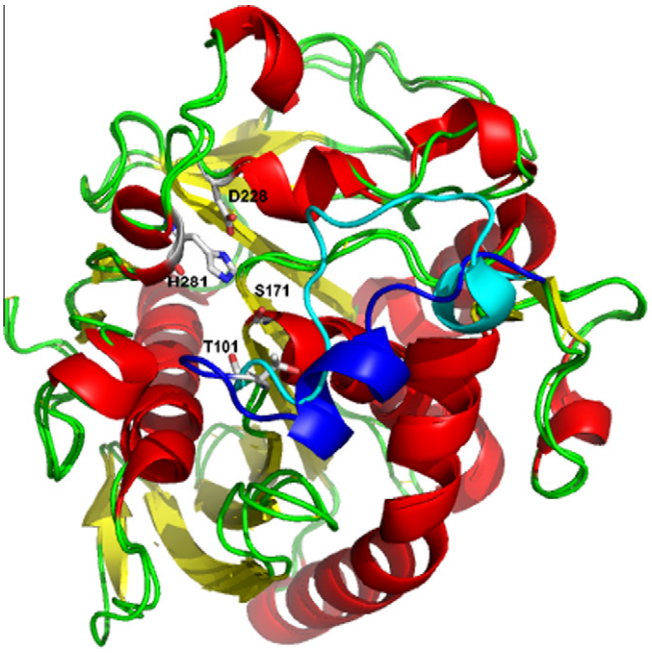

Fig. 1. Superimposition of the constructed structure with the crystal structure. The lid domain of crystal and constructed structure was highlighted in cyan and blue, respectively. In the constructed structure, the lid adopted a form of a typical α-helix, and catalytic sites were exposed to solvent. Compared with the crystal structure, Thr101 was brought to a considerable different position and allowed the formation of oxyanion hole (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

brought to a proper position, at which Thr101 and Leu172 localized a canonical oxyanion hole (Fig. 1).

Moreover, the Ramachandran plot of the constructed structure and the crystal structure are almost identical, with Glu61 and Asp245 in the disallowed regions, which has been observed for the crystal structure as well. Therefore, the constructed open structure adopting the amphiphilic α-helix in the lid domain is reliable.

3.2. The configuration of analogue and intact hydrogen bonds network

A lipase structure in complex with the phosphonate, a substrate analogue, represents the putative transition-state conformation of a substrate bound to the active site. In the X-ray crystal studies of lipases inhibited by phosphonate, although the racemic mixture of the inhibitor was used in the crystallization, only one phosphorus enantiomer, Sp was observed in the crystal structures [18,19,22]. In the structure of SMG1-analogue complex optimized by MD, the configuration of analogue was in agreement with the observation described above (Fig. 2B).

As shown in the Fig. 2B, the analogue was covalently linked at its phosphorus atoms to the nucleophilic O_γ of Ser171 and accommodated into the hydrophobic active site clefts. The oxygen of P = O settled into the oxyanion hole, and therefore hydrogen bonds to the backbone nitrogen atoms of Thr101 and Leu172 were easily formed. The phosphonyl oxygen and the O_γ of Ser171 were approximately equidistant, ~3 Å, from the Nε2 of His281 in an arrangement of a typical bifurcated hydrogen bond. Together with two hydrogen bonds formed from Asp228 to Nδ1 of His281, these intact hydrogen bonds are of functional importance for catalysis and could be observed in the SMG1-analogue complex optimized by MD. These results suggested that this structural model was practical in analysis of the binding model of this lipase.

3.3. Binding model of SMG1in the open conformation

In the optimized structure of SMG1-analogue complex (Fig. 2A), Leu103 and Phe278 divided the catalytic pocket into two separated moieties, an exposed groove (acyl moiety) and a narrow tunnel

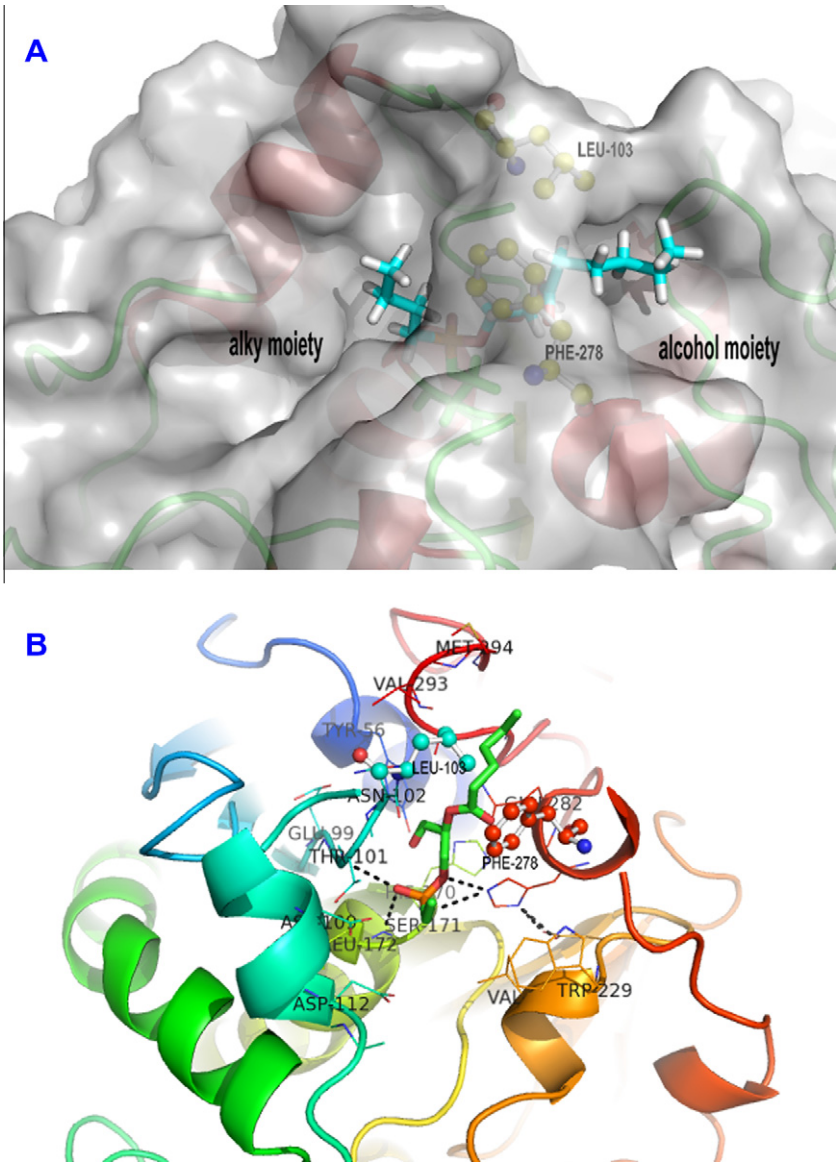

Fig. 2. Structural model of substrate analogue bound to active site of SMG1. (A) Leu103 and Phe278 divided the whole catalytic into two seperated moieties, an exposed groove (alky moiety) and a narrow tunnel (alcohol moiety). The *sn-3* alkyl chain lain in this exposed groove on the left side and the *sn-2* moiety snugly fitted in the tunnel. (B) Residues involved in the catalytic sites and binding model were labeled, and hydogen bonds of functional importance were shown in black dash. The configuration of diacylglycerol analogue at phosphorus atom, Sp, is highly conserved in crystal structure of lipases.

(glycerol moiety). The *sn-3* alkyl chain lain in the exposed groove lined by Asp109, Asp112, Ala113, Phe115, Leu172, Trp229, Val233, Pro234, Pro235, and Phe278. This groove was so large that it would accommodate a fatty acid of length of 12, giving rise to no contacts. And the *sn-2* moiety of substrate inserted into the narrow, hydrophobic tunnel, which was encompassed by Asn102, Leu103, His170, Phe278, Ala292, Val293, and Met294. The *sn-2* moiety fitted snugly in the tunnel. The interactions between substrate analogue and SMG1 were mostly of a hydrophobic nature.

3.4. The substrate selectivity mechanism of SMG1

Among the lipases of known 3D structure, SMG1 has significant homology in structure with the RML in the closed conformation (RMSD = 1.57 Å for main chain). Therefore, we constructed the open, active conformation model using the open state of RML as template (Fig. 1).

SMG1 is identified to be specific for mono- and diacylglycerol but not triacylglycerol, while RML is a typical lipase active on triacylglycerol. Structural comparison between SMG1 and RML in the open conformation showed that a striking difference occurred in the beginning of the lid domain, largely owing to a bulk, hydrophobic two-residue (Leu103 and Phe104) insertion in SMG1 (Fig. 3). Moreover, Leu103 together with residue at the opposite side, Phe278, encompassed the tunnel and separated it from the exposed groove (Fig. 2A). However, in the case of RML, Val254, located at a corresponding position of Phe278 in SMG1, projected its side chain towards protein surface rather than towards catalytic center. Through these modifications, the shape and size glycerol moiety binding site, varied considerably between SMG1 and RML, and the tunnel-shaped organized in SMG1 was replaced by a wider crevice-shaped structure in RML. Therefore, these allowed RML to offer substrates broader access to the active site compared to SMG1.

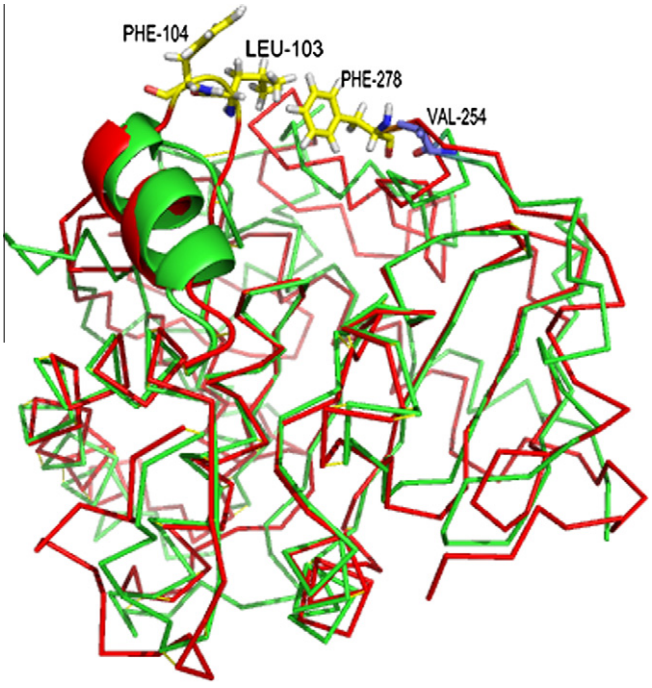

Fig. 3. Superimposition of Cα trace of SMG1 and RML. The structure of SMG and RML was colored red and green, respectively, and the lid domain was highlighted in the form of cartoon. Compared with RML, there was a two-residue (Leu103 and Phe104) insertion in SMG1. Phe278 in SMG1 was oriented towards catalytic center, while in RML, the corresponding residue, Val254, projected towards protein surface (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Now that the exposed groove is large enough, could it accommodate the third chain of triacylglycerol? As already pointed out, the configuration of substrate analogue at phosphorus atom is highly conserved in lipases, and these geometric restrictions would situate the third chain at the opposite direction of this groove, meaning that the third chain can only settle into the narrow tunnel. However, this tunnel has been completely occupied by *sn-2* so that the third chain cannot insert in. Therefore, SMG1 is strictly specific for mono- and diacylglycerol, but not triacylglycerol.

In view of the striking differences of the substrate binding model in the alcohol binding moiety, it is not surprisingly that SMG1 and RML differ completely in their substrate selectivity. RML can efficiently hydrolyze the triacylglycerol, while SMG1 is strictly specific for mono- and diacylglycerol. The structure of SMG1-analogue suggested that Leu103 and Phe278 play an important role in conferring this selectivity by encompassing the tunnel which is restricted to a fatty acid chain fitted in only. In SMG1, Phe278 was oriented towards catalytic center while in RML, Val254, equivalent to Phe278, projected towards protein surface. Together with the two-residue insertion in SMG1, these differences can explain that SMG1 and RML share high structural similarity but they are quite distinct in substrate selectivity.

Acknowledgments

We would like to thank Ruoxu Gu for his instructive advice and useful suggestions in the completion of this paper. This work was supported by Program for New Century Excellent Talents in University (NCET-11-0160), Fundamental Researh Funds for the Central Universities (2011ZZ0018), and Guangzhou Key Technology R&D Program (2009A1-E021).

References

[1] D.L. Ollis, E. Cheah, M. Cygler, et al., The alpha/beta hydrolase fold, Protein Eng. 5 (1992) 197–211.
[2] S. Yamaguchi, T. Mase, Purification and characterization of mono- and diacylglycerol lipase from *Penicillium camembertii U-150*, Appl. Microbiol. Biotechnol. 34 (1990) 720–725.
[3] M. Karlsson, J.A. Contreras, U. Hellman, et al., CDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases,, J. Biol. Chem. 272 (1997) 27218–27223.
[4] T. Sakiyama, T. Yoshimi, A. Miyake, et al., Purification and characterization of a monoacylglycerol lipase from *Pseudomonas sp. LP7315*, J. Biosci. Bioeng. 91 (2001) 27–32.
[5] G. Labar, C. Bauvois, F. Borel, et al., Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling, ChemBioChem 11 (2010) 218–227.
[6] Y.M. DeAngelis, C.W. Saunders, K.R. Johnstone, et al., Isolation and expression of a *Malassezia globosa* lipase gene, LIP1, J. Invest. Dermatol. 127 (2007) 2138–2146.
[7] W. Wang, T. Li, X. Qin, et al., Production of lipase SMG1 and its application in synthesizing diacylglyecrol, J. Mol. Catal. B-enzym. 77 (2012) 87–91.
[8] D. Xu, L. Sun, H. Chen, et al., Enzymatic Synthesis of Diacylglycerols Enriched with Conjugated Linoleic Acid by a Novel Lipase from *Malassezia globosa*, J. Am. Oil Chem. Soc. (2012), http://dx.doi.org/10.1007/s11746-012-2018-x.
[9] T. Xu, L. Liu, S. Hou, et al., Crystal structure of a mono- and diacylglycerol lipase from *Malassezia globosa* reveals a novel lid conformation and insights into the substrate specificity, J. Struct. Biol. 178 (2012) 363–369.
[10] U. Derewenda, L. Swenson, R. Green, et al., An unusual buried polar cluster in a family of fungal lipases, Nat. Struct. Biol. 1 (1994) 36–47.
[11] K. Isobe, K.D. Aumann, R.D. Schmid, A structural model of mono- and diacylglycerol lipase from *Penicillium camembertii*, J. Biotechnol. 32 (1994) 83–88.
[12] J.A. Cuff, M.E. Clamp, A.S. Siddiqui, et al., JPred: a consensus secondary structure prediction server, Bioinformatics 14 (1998) 892–893.
[13] C. Carrasco-López, C. Godoy, B. de Las Rivas, et al., Activation of bacterial thermoalkalophilic lipases is spurred by dramatic structural rearrangements, J. Biol. Chem. 284 (2009) 4365–4372.
[14] K.K. Kim, H.K. Song, D.H. Shin, et al., The crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* reveals a highly open conformation in the absence of a bound inhibitor, Structure 15 (1997) 173–185.
[15] N. Eswar, B. Webb, M.A. Marti-Renom, et al., Comparative protein structure modeling using MODELLER, Curr Protoc Protein Sci. 50 (2007) 2.9.1–2.9.31.

[16] U. Derewenda, A.M. Brzozowski, D.M. Lawson, Z.S. Derewenda, Catalysis at the interface. The anatomy of a conformational change in a triglyceride lipase, Biochemistry 31 (1992) 1532–1541.
[17] M. Nardini, D.A. Lang, K. Liebeton, et al., Crystal structure of *pseudomonas aeruginosa* lipase in the open conformation. The prototype for family I.1 of bacterial lipases, J. Biol. Chem. 275 (2000) 31219–31225.
[18] D.A. Lang, M.L. Mannesse, G.H. de Haas, et al., Structural basis of the chiral selectivity of *Pseudomonas cepacia* lipase, Eur. J. Biochem. 254 (1998) 333–340.
[19] P. Grochulski, F. Bouthillier, R.J. Kazlauskas, et al., Analogs of reaction intermediates identify a unique substrate binding site in *Candida rugosa* lipase, Biochemistry 29 (1994) 3494–3500.
[20] J. Uppenberg, N. Ohrner, M. Norin, et al., Crystallographic and molecular-modeling studies of lipase B from *Candida antarctica* reveal a stereospecificity pocket for secondary alcohols, Biochemistry 26 (1995) 16838–16851.
[21] U. Derewenda, L. Swenson, Y. Wei, et al., Conformational lability of lipases observed in the absence of an oil–water interface. Crystallographic studies of enzymes from the fungi *Humicola lanuginosa* and *Rhizopus delemar*, J. Lipid Res. 35 (1994) 524–534.
[22] M.P. Egloff, F. Marguet, G. Buono, et al., The 2.46 Å resolution structure of the pancreatic lipase–colipase complex inhibited by a C11 alkyl phosphonate, Biochemistry 7 (1995) 2751–2762.