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Computer-aided modelling of stereoselective triglyceride hydrolysis catalyzed by *Rhizopus oryzae* lipase

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Abstract

Lipase from *Rhizopus oryzae* catalyzes the stereoselective hydrolysis of triglycerides and analogues. Stereopreference and degree of enantiomeric excess of the product varies with the structure of the substrate: trioctanoylglycerol ('ester') and the *sn*-2 analogues 2-X-1,3-dioctanoylpropandiol, where 2-X = 2-*O*-octyl ('ether') and 2-hexyl ('alkane'), are preferentially hydrolyzed at *sn*-1, substitution by 2-phenyl ('phenyl') reverses the stereopreference to *sn*-3.

We have modelled the stereoselectivity of *Rhizopus oryzae* lipase by docking the tetrahedral intermediates of these substrates in two orientations, appropriate to hydrolysis at *sn*-1 or *sn*-3, respectively. The initial complexes were further relaxed by molecular dynamics simulations. The favoured orientation of a substrate is characterized by three factors: (1) The substrate fits well into the binding site. The glycerol backbone is relaxed and the *sn*-2 chain points to a well defined hydrophobic binding site. (2) The oxyanion is stabilized by an extra hydrogen bond from the side chain of Thr 83. (3) The substrate lacks repulsive interactions with protein side chains, especially of Leu 258.

Our model is consistent with experimental data and explains qualitatively the ranking of four different substrates with regard to stereoselectivity. It can be used to design lipase mutants with modified stereoselectivity.

Keywords: Microbial lipases; *Rhizopus oryzae* lipase; Modelling; Stereoselective hydrolysis

1. Introduction

Lipases (triacylglycerol hydrolase, EC 3.1.1.3) are serine hydrolases and catalyze the hydrolysis of organic esters as well as esterification [1]. For 12 lipases, the structure has been solved at high resolution (see [2] for a review).

The natural substrates of lipases are triglycerides, but they also accept other hydrophobic esters of primary and secondary alcohols. One important property of lipases, their stereoselectivity with respect to stereocenters in the alcohol moiety, was successfully applied to the preparation of optically pure secondary alcohols [3]. An empirical rule was proposed to predict which

Abbreviations: ROL, *Rhizopus oryzae* lipase; RDL, *Rhizopus delemar* lipase; RML, *Rhizomucor miehei* lipase; PDB, Protein Data Bank (Brookhaven)

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enantiomer reacts faster [4]. The mechanism of stereoselectivity was confirmed by X-ray structure analysis for *Candida rugosa* lipase [5] and generalized to other lipases [2]. For chiral or prochiral primary alcohols like triglycerides it was demonstrated for a variety of substrates that lipases can also distinguish between the enantiomers [6,7]. In contrast to secondary alcohols, however, there is no general rule to predict stereopreference [8]. Several modelling studies have investigated the mechanism of triglyceride binding by lipases [9–12]. The molecular mechanism causing stereopreference and the influence of substrate structure is not yet understood.

We have based our investigation on an experimental study of *Rhizopus oryzae* lipase (ROL): Stadler et al. [7] observed that ROL is highly regioselective for the (1,3) position in triglycerides, and the enantiomeric excess of the resulting diglycerides depends on the chemical structure of the *sn*-2 substitution: upon substitution of the ester group by an ether group, the selectivity for *sn*-1 increases, while for a phenyl substituent the selectivity reverses to *sn*-3. They concluded that the interaction of the substrates' *sn*-2 moiety with the lipase determines stereoselectivity, while chain length is of minor importance. They also compared the hydrolysis of rac-1-*O*-alkyl-2,3-dioleoylglycerol and rac-1,2-*O*-dialkyl-3-oleoylglycerol and found a similar selectivity as for trioleoylglycerol and 1,3-dioleoyl-2-*O*-alkylglycerol. From that they concluded that the non-hydrolyzed (3,1) chain does not influence the selectivity significantly.

We have used computer-aided molecular modelling to explain these experimental findings and to identify the molecular interactions which lead to the experimentally observed properties, thereby trying to answer the following questions:

(1) Why is ROL *sn*-1 selective for ether, alkane and ester substrates?

(2) What causes the quantitative difference in selectivity towards ether and ester?

(3) Why is selectivity reversed to *sn*-3 for phenyl substrates?

2. Methods

2.1. Molecular dynamics simulations

The structure of ROL in the open form was modelled previously based on its homology to *Rhizomucor miehei* lipase (RML), and a triglyceride molecule was docked to its binding site [12]. We modified the triglyceride substrate to the ether, alkane and phenyl analogues to investigate the different stereoselectivity towards these substrates. The complex was relaxed by molecular dynamics simulations. To reduce the simulation time we built a 'miniprotein' consisting of all amino acids with at least one atom at less than 3 Å distance from the substrate. The resulting stretches of amino acids were further elongated by one or two additional amino acids to reduce the interaction of terminal charge with the substrate. The final 'miniprotein' consists of 100 amino acids of the 265 residues of ROL. During energy minimization and molecular dynamics simulations we applied position constraints to the enzyme backbone. The side chains and the substrate were allowed to move. The simulations were performed in vacuo. Molecular dynamics simulations of the complex of all four substrates each in *sn*-1 and *sn*-3 orientation were carried out using Sybyl 6.1 (Tripos Inc.) with the Tripos force field [13]. For the catalytic histidine and the oxyanion the partial charges were modified (Fig. 1) as calculated by the semi-empirical method MNDO94/PM3 [14] using the Unichem 3.0 interface (Cray Research Inc.).

The complexes were gradually heated in 4 intervals of 2 ps each to 5, 30, 100, 200 K and during 6 ps to 300 K. Step size was 1 fs up to 100 K and 0.5 fs at 200 and 300 K. The temperature coupling constant was set to 10 fs [15], the non-bonded interaction cut-off to 8 Å. After this equilibration phase the system was further simulated for 4 ps. Structure and torsion angles were averaged only during this production phase. We performed three simulation runs for each enzyme–substrate complex with ran-

dom initial velocities to reduce the influence of initial conditions on the conformation of the complex.

2.2. Energy of the substrate

The energy of the average substrate conformation was evaluated by constrained geometry optimization of a 'mini-substrate' using MNDO94/PM3. The 'mini-substrate' consists of the glycerol backbone and the first three atoms of the *sn*-2 moiety. Torsion angles were adjusted to the average values of the substrate. The geometry of the 'mini-substrate' was optimized with constrained torsion angles.

2.3. Interaction of the substrate with ROL

To predict putative fatty acid binding sites, GRID96 [16] was used. This program searches for binding sites of a protein by scanning the protein surface by probes with different properties. We used the 'METHYL' and the yet undocumented 'DRY' probe (P.J. Goodford, personal communication) to search for hydrophobic binding sites.

To determine the amino acids which mediate stereoselectivity, we investigated the influence of substrate orientation on side chain geometry. The average structures of a complex with the substrate in *sn*-1 and *sn*-3 orientation were superposed and the geometry of the side chains was compared. For most of the side chains the geometry was independent of substrate orientation. The few exceptions with different geometry were identified and analyzed in more detail.

3. Results

3.1. Modelled ROL structure

We compared our modelled ROL structure with the experimental structures of the open form of the homologous *Rhizomucor miehei* lipase (RML, [17]) and *Rhizopus delemar* lipase (RDL, [18]). A comparison of 19 amino acids interacting with the substrate shows a C_α rms deviation of 0.5 Å to RDL and 0.3 Å to RML. In the lid region, the differences are slightly larger, which can be explained by the high flexibility of this region [19].

3.2. Binding of substrates

The binding site of ROL can be divided into three regions: (1) the catalytic serine spot, (2) a long and deep hydrophobic crevice of about 2×16 Å (determined by the GRID 'METHYL' probe at a potential of -0.15) and (3) a shallow hydrophobic dent of about 2×5 Å (determined by the GRID 'DRY' probe at a potential of -0.01) parallel to the hydrophobic crevice (Fig. 2). Which is the most probable conformation of the bound substrates? The starting point for generation of the substrates' conformation is the position of the polarized ester group of the tetrahedral intermediate. Its orientation in the binding site is derived by analogy from the experimentally determined structure of RML

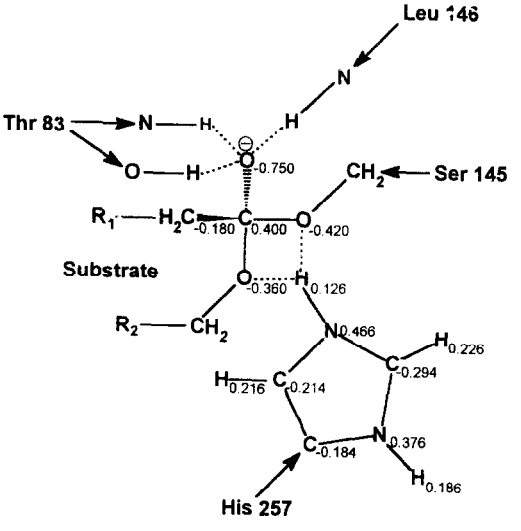

Fig. 1. Schematic view of the active site: side chains of the catalytic Ser 145 and His 257, the oxyanion hole formed by the backbone of Leu 146 and Thr 83 and the side chain of Thr 83. A substrate is bound as tetrahedral intermediate. The acid and alcohol moieties of the substrate are indicated as R_1 and R_2, respectively. Partial charges for each atom are given as fractional electronic unit charge.

with an inhibitor bound covalently to the catalytic serine [12]. The direction of the alcohol moiety is given by the position of the catalytic histidine. The acyl chain of the fatty acid which is hydrolyzed (*sn*-1 or *sn*-3, depending on the substrate's orientation) points towards the hydrophobic crevice. For substrates with an aliphatic chain in *sn*-2 position (ester, ether and alkane), the most probable binding site for the *sn*-2 chain is the hydrophobic dent (Fig. 3 a,b for *sn*-1 and *sn*-3 orientation, respectively). The two chains will then be parallel and contact each other at a C–C distance of about 4 Å, thereby increasing mutually their hydrophobic contact area. This co-operative effect stabilizes the complex. The third chain of a triglyceride binds to the two other chains and only to a minor extent to the enzyme. We call this binding mode '*sn*-2 down'.

For triglycerides and analogues there is an alternative binding mode with the non-hydrolyzed *sn*-3(1) chain binding to the hydrophobic dent (Fig. 3 c,d), and the *sn*-2 chain bound to the 1,3 chains ('*sn*-2 up'). The two binding modes '*sn*-2 down' and '*sn*-2 up' are not equivalent. In the '*sn*-2 down' mode the hydrophobic acyl chains of the two non-hydrolyzed fatty acids pack favourably to each other (Fig. 3 a,b) as do the polar ester groups. In the '*sn*-2 up' binding mode the polar ester group of the non-hydrolyzed *sn*-1(3) chain contacts the two other hydrophobic chains (Fig. 3 c,d). Therefore, for

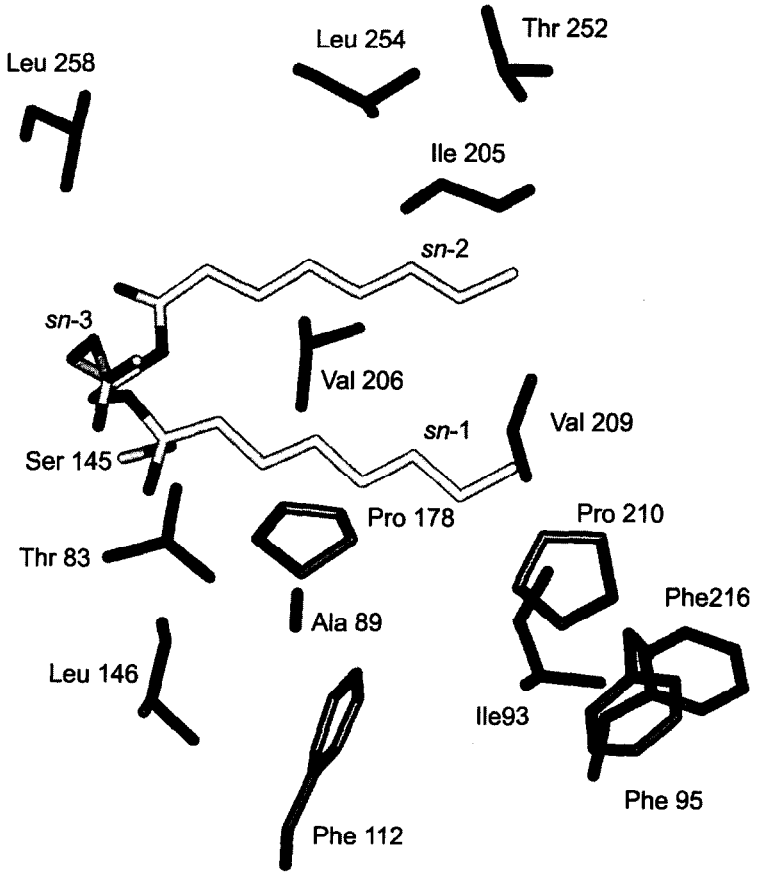

Fig. 2. An ester substrate (carbon atoms, white; oxygen atoms, black) docked to the ROL binding site in the *sn*-1 orientation. The non-hydrolyzed *sn*-3 chain is not visualized. The binding sites are determined by GRID. Residues of the hydrophobic crevice (dark grey): Thr 83, Leu 146, Val 206, Pro 178, Ala 89, Phe 112, Val 209, Pro 210, Ile 93, Phe 95, Phe 216; residues of the hydrophobic dent (black): Leu 258, Leu 254, Ile 205, Thr 252. The entrance to the binding pocket is on the right, the catalytic Ser 145 on the left.

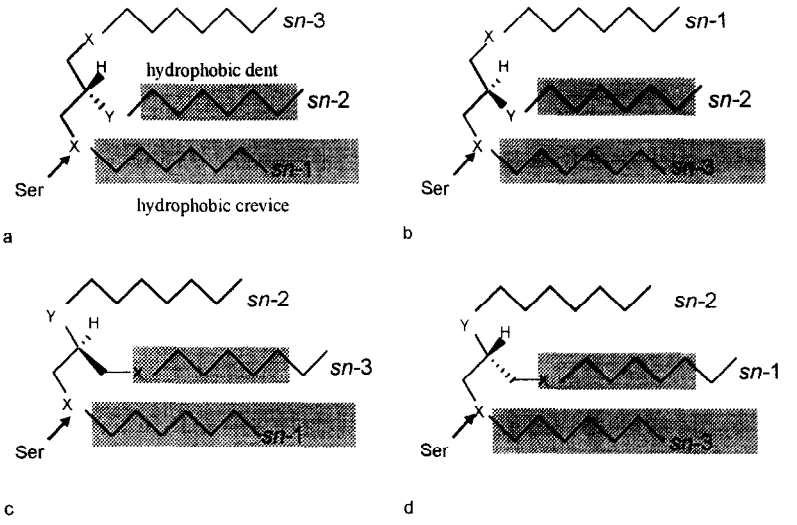

Fig. 3. Four possibilities of binding for a substrate to ROL. The long and the short grey shaded regions indicate the hydrophobic crevice and the hydrophobic dent, respectively. The catalytic serine marks the cleavage site. The *sn*-2 chain is highlighted, X corresponds to the ester bond in the *sn*-1 and the *sn*-3 chain. Y denotes the different *sn*-2 groups of ether, ester or alkane. In the phenyl substrate, Y and the aliphatic chain are replaced by a phenyl ring. The substrate can bind in the *sn*-1 (a,c) or the *sn*-3 (b,d) orientation. In the '*sn*-2 down' binding mode (a,b), the *sn*-2 chain binds to the hydrophobic dent, in '*sn*-2 up' (c,d), it does not bind to the lipase.

substrates with an aliphatic *sn*-2 chain the '*sn*-2 up' binding mode should be less favourable than the '*sn*-2 down' mode. Molecular dynamics simulations of ether, ester and alkane substrates in '*sn*-2 up' binding mode support this intuitive prediction, since the non-hydrolyzed *sn*-1(3) chain moved out of the hydrophobic dent in 6 out of 8 simulations, while the complex was stable in the '*sn*-2 down' binding mode. For the phenyl substrate, however, the '*sn*-2 up' binding mode is more favourable. The non-hydrolyzed *sn*-3(1) aliphatic chain binds to the hydrophobic dent, and the phenyl group points out of the binding site.

3.3. *sn*-1 preference of ether, ester and alkane

Upon binding to the lipase, the substrate adapts itself to the binding site. Since the configuration at glycerol C2 is opposite in *sn*-1 and *sn*-3 orientation of the substrate, the torsion angles of the glycerol backbone have to adjust to allow the *sn*-2 chain to bind to the hydrophobic dent. Therefore, the glycerol conformation is different in the *sn*-1 and *sn*-3 orientations. We considered only the *sn*-2 down binding mode for the reasons mentioned above. Two effects were identified which favour the *sn*-1 orientation. The torsion angles of the glycerol backbone were averaged for three molecular dynamics runs for each of the substrates in both orientations, and the conformational energy was evaluated (Fig. 4). From the difference of 4–5 kcal/mol in favour of the *sn*-1 orientation, we conclude that the substrates bind preferentially in the *sn*-1 rather than in *sn*-3 orientation.

A second interaction also favours the *sn*-1 orientation. For all three substrates in *sn*-1 ori-

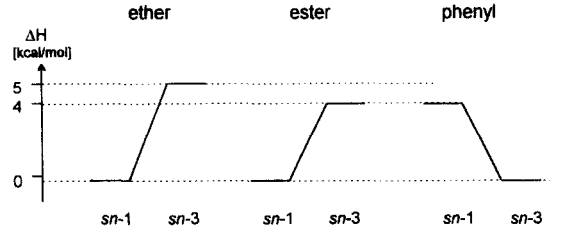

Fig. 4. Energy differences of the glycerol backbone for the two orientations. Energies were calculated semi-empirically using a 'mini-substrate' (see Methods). Ether and ester substrates are bound in the '*sn*-2 down', the phenyl substrate in the '*sn*-2 up' mode.

entation, the side chain hydroxyl of Thr 83 forms a hydrogen bond to the oxyanion (Fig. 5 a). In *sn*-3 orientation it moves away, which may be caused by repulsive interaction of the hydroxyl group with glycerol C3. Instead of stabilizing the oxyanion, the hydroxyl of Thr 83 then points to Asp 92. Thus, the transition state is less stabilized, which leads to a slower reaction of the substrate in the unfavoured *sn*-3 orientation.

3.4. Difference in stereoselectivity of ether and ester

There is clear experimental evidence that ROL hydrolyzes more selectively an ether than

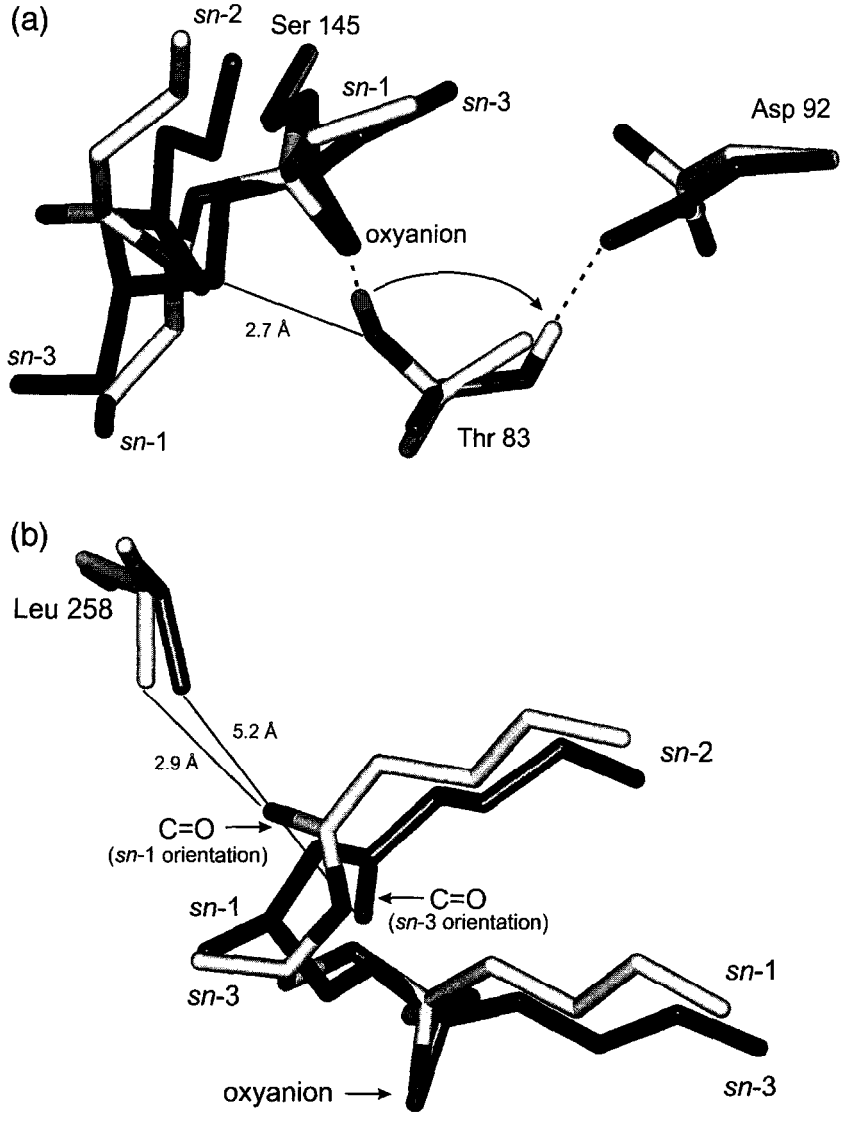

Fig. 5. (a) Orientation of Thr 83 side chain for the ester substrate in the *sn*-1 (light grey) and the *sn*-3 (dark grey) orientations (oxygen atoms, black). In the *sn*-1 orientation, the oxyanion is stabilized by an additional hydrogen bond (dotted line). In the *sn*-3 orientation, the glycerol C3 would contact the Thr 83 hydroxyl group (solid line, C-O distance 2.7 Å), if the hydroxyl group pointed towards the oxyanion. Therefore, the side chain moves towards Asp 92. (b) The *sn*-2 carbonyl group of the ester substrate in the *sn*-1 (light grey) and the *sn*-3 (dark grey) orientation. The hydrophobic side chain of Leu 258 contacts the carbonyl oxygen, if the substrate is bound in the *sn*-1 orientation.

an ester substrate. So we concluded that there should be either an additional stabilizing interaction of ester in the *sn*-3 orientation or a destabilizing interaction in the *sn*-1 orientation. This interaction should be local, since the only differences between the two substrates are an additional oxygen atom in the ester substrate and the rigidity of the ester group. Therefore, we compared the geometry of the *sn*-2 ester group in both orientations. The binding geometry of the substrate in the two orientations is governed by a pseudo-mirror symmetry (Fig. 5 b), which is the consequence of the similar position of the glycerol C2, the different direction of the C2–O bond, and the similar position of the *sn*-2 fatty acid chains in the hydrophobic dent. However, the protein environment is not symmetric, so there are different interactions in the two orientations. The major effect is a steric repulsion between the side chain of Leu 258 and the *sn*-2 carbonyl oxygen of the substrate in *sn*-1 orientation. In addition, the carbonyl oxygen is located near a hydrophobic site. Since in *sn*-3 orientation, it does not interact with ROL, this interaction favours the *sn*-3 orientation. This situation is different for the ether substrate, since no essential difference in local interactions can be found in the two orientations.

3.5. Phenyl substrate

The chemical structure of this substrate's *sn*-2 site differs from the three other substrates in two aspects: the phenyl ring is large and rigid and there is no aliphatic *sn*-2 chain. As a consequence, compared to the three other substrates with aliphatic chains, the substrate will probably bind to ROL in the '*sn*-2 up' binding mode (Fig. 3 c,d). In this binding mode the *sn*-3 orientation is favoured by three effects: (1) The glycerol backbone angles of phenyl substrate in *sn*-3 and *sn*-1 orientation are similar to the respective angles of the three other substrates in the *sn*-1 and *sn*-3 orientations, respectively. This is reflected in the difference in energy of 4 kcal/mol favouring the *sn*-3 orientation of the phenyl substrate (Fig. 4). (2) In the *sn*-3 orientation, the oxyanion is stabilized by a Thr 83 hydrogen bond. (3) In the *sn*-1 orientation, there is a repulsive interaction between the phenyl

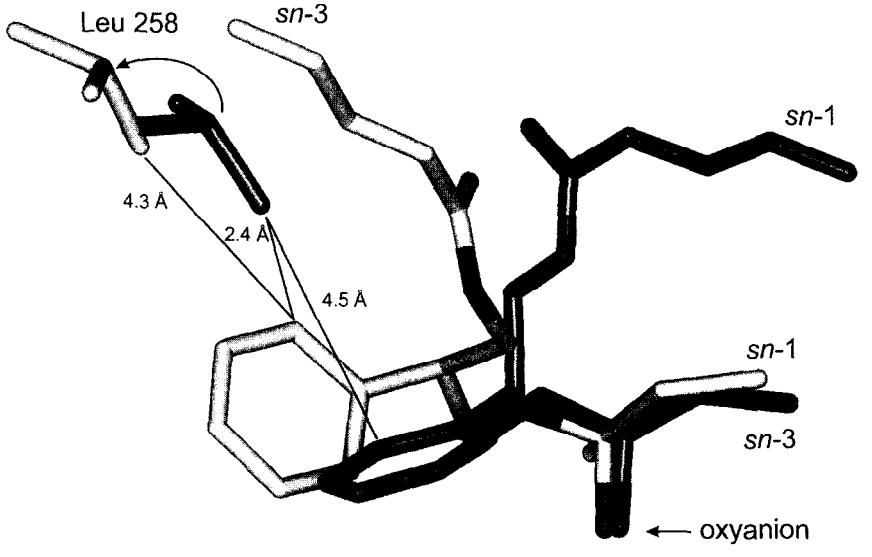

Fig. 6. The phenyl substrate in the *sn*-1 (light grey) and the *sn*-3 (dark grey) orientation. A clash (solid line, C–C distance 2.4 Å) would occur between Leu 258 and the phenyl ring, if the substrate was in the *sn*-1 orientation (light grey) and the Leu 258 side chain (dark grey) in the conformation appropriate to binding the substrate in the *sn*-3 orientation. Therefore, the side chain of Leu 258 moves away upon binding of phenyl substrate in the *sn*-1 orientation.

ring and the side chain of Leu 258, which does not occur in the *sn*-3 orientation (Fig. 6)

4. Discussion

Numerous experiments have demonstrated that lipases stereoselectively hydrolyze organic esters. Several factors have been assigned to determine the degree of stereoselectivity of serine hydrolases. For lipase-catalyzed hydrolysis of secondary alcohol esters, X-ray structure determination has clearly demonstrated the structural basis of stereoselectivity [5]. However, detergent and organic solvent were also suggested as factors either by direct interaction with the enzyme–substrate complex [20] or indirectly by modulation of enzyme flexibility [21].

In our study, we focus on the structural basis of stereoselectivity of ROL towards triglycerides and analogues. While the backbone is constrained, protein side chains and the substrate are allowed to relax, since limited relaxation has been shown to be essential to discriminate between enantiomers in proteases [22].

4.1. Binding site

The two most prominent binding sites of ROL are two long parallel hydrophobic patches, which start near the active site serine and direct towards the entrance to the ROL binding site. One of these, the hydrophobic crevice, is comparable to hydrophobic sites which were previously described in modelling studies of the structurally similar lipases from *Rhizomucor miehei* and *Humicola lanuginosa* [9]. A more detailed analysis [10] identified an additional hydrophobic site in *Humicola lanuginosa* lipase, which corresponds to the hydrophobic dent in ROL. For three lipases, experimental data are available how fatty acid chain analogues bind to the lipase. In the lipases from *Candida antarctica* [23] and *Candida rugosa* [24], the binding site for the hydrolyzed acyl chain has been identified, in human pancreatic lipase [25] there is a second, distinct binding site for an acyl chain. Since the structures of these lipases are different from ROL, it has to be further investigated whether our concept can be generalized.

In ROL there are two hydrophobic binding sites, thus the three chains of a triglyceride substrate or an analogue will compete for binding. For hydrolysis at the 1,3 position, there are four ways how a substrate can bind. We distinguish two orientations (leading to cleavage at *sn*-1 or *sn*-3) and two binding modes (*sn*-2 chain binding to the hydrophobic dent or not). Our structural model of the complex can be used to analyze for a given substrate, which of the four possibilities is the most probable one, and thus to predict stereoselectivity.

In the case of substrates with an aliphatic chain in *sn*-2 (ester, ether and alkane substrate), our model predicts that the '*sn*-2 down' binding mode is preferred: the hydrolyzed chain and the *sn*-2 chain interact with the hydrophobic dent, while the non-hydrolyzed *sn*-3(1) chain binds to the two already bound chains.

4.2. Factors determining stereoselectivity

Stereoselectivity is primarily determined by the chemical structure of the *sn*-2 chain, and only to a minor degree by the *sn*-3(1) chains, as has already been concluded from experimental observations: (1) The two binding modes (Fig. 3) are not equivalent, since stereopreference is experimentally observed [6,7,26]. (2) This model is further confirmed by comparing the enantiomeric excess of hydrolyzed rac-1-*O*-alkyl-2,3-dioleoylglycerol and rac-1,2-*O*-dialkyl-3-oleoylglycerol [7]. The stereoselectivity towards the substrate with ester bond in *sn*-2 is similar to the value for triglyceride, while the value for the ether analogue corresponds to 2-*O*-alkyl-1,3-diacylglycerol. (3) 2-*O*-alkyl analogues of rac-1,2(2,3)-diacylglycerols are preferentially hydrolyzed at *sn*-1, like the triglyceride analogue [27]. These experiments demonstrate that the non-hydrolyzed *sn*-3/1 chain has only a

minor influence on stereopreference. This situation changes for the phenyl substrate. Here the '*sn*-2 up' binding mode is favoured, with the two *sn*-1(3) acyl chains binding to the lipase and the phenyl ring pointing out of the binding site.

For all substrates in the '*sn*-2 down' binding mode (ether, alkane and ester), the *sn*-1 orientation is preferred to the *sn*-3 orientation by two factors: (1) The glycerol backbone is less disturbed by the ROL binding site, thus its internal energy is lower. (2) The oxyanion is stabilized by an additional hydrogen bond from the side chain hydroxyl group of Thr 83. This residue has already been suggested to act as hydrogen donor to the tetrahedral intermediate of the substrate [17]. The role of this hydroxyl group was further investigated by protein engineering. Upon replacing Thr 83 by Ser, the activity of the ROL mutant enzyme is still 22% of the wild type. Removing the hydroxyl group by replacing Thr 83 by Val or Ala reduces the activity to less than 0.1% [12,28].

The general preference of the *sn*-1 orientation, however, is modulated by interactions of the *sn*-2 group with protein side chains. Since alkane and ether are small and flexible, they fit well into the protein without interfering with side chains, while the carbonyl group of the ester substrate sticks out of the chain, which might lead to repulsion. In our model, the direction from glycerol C2 to the *sn*-2 chain is different for both orientations, therefore the carbonyl group interacts differently in both orientations. In the *sn*-1 orientation, the carbonyl oxygen is in contact with the hydrophobic Leu 258 side chain, while in the *sn*-3 orientation it is not in contact with lipase. This observation can explain the reduced *sn*-1 preference of the ester substrate, which has been observed experimentally [7]. The model can also be used to predict mutation sites which mediate stereoselectivity towards the ester substrate. For the phenyl substrate, the situation is similar: the bulky and rigid phenyl ring interacts differently with protein side chains. The *sn*-3 orientation is preferred, while in *sn*-1 orientation the phenyl ring interferes with Leu 258.

4.3. Role of solvent

Stereoselectivity results from the equilibrium between four possibilities, how a triglyceride or analogue binds to ROL. Small changes of less than 1 kcal/mol in free energy of binding (as calculated from $\Delta\Delta G = -RT \ln E$) are sufficient to reverse stereopreference. These changes could be introduced by the presence of organic solvent, detergent or emulsifier, which could influence structure or flexibility of either substrate or enzyme [20]. Based on our modelling studies, we propose a third possibility: even at low concentration, detergent and organic solvent molecules can bind to the hydrophobic binding sites of the enzyme and thus influence the balance. This effect will also depend on the properties of the binding sites in different lipases.

4.4. Comparison to esters of secondary alcohols

For esters of secondary alcohols, the site determining stereoselectivity is an integral part of the active site. Binding of the substrate and selectivity towards the alcohol moiety is mediated by a small and a large pocket [2]. Since the catalytic triad is identical in all lipases and the mechanism of hydrolysis is identical for all substrates, the rule for stereopreference is generally valid.

In triglycerides, both the stereocenter and the dominating binding sites are located further away from the active site. The geometry of the alcohol binding site and the location of the two hydrophobic acyl chain binding sites restrict the conformational freedom of the bound substrate. Upon binding of the acyl chains, the glycerol backbone is pressed against the protein, thereby deforming both substrate and protein side chains. The degree of repulsive interaction depends on the orientation of the substrate and the structure of the *sn*-2 chain. This basic difference between

triglycerides and esters of secondary alcohols explains the considerable variability of stereoselectivity towards triglycerides. For triglycerides, this uncoupling of catalytic mechanism and stereoselectivity opens the door to design fully active mutants with changed stereoselectivity.

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References

[1] L. Alberghina, R.D. Schmid and R. Verger (Eds.), Lipases: Structure, Mechanism and Genetic Engineering, VCH Publishers, New York, 1991.
[2] R.J. Kazlauskas, Trends Biotechnol., 12 (1994) 464–472.
[3] K. Faber, Biotransformation in Organic Chemistry, Springer Verlag, Weinheim, 1992.
[4] R.J. Kazlauskas, A.N.E. Weissfloch, A.T. Rappaport and L.A. Cuccia, J. Org. Chem., 56 (1991) 2656–2665.
[5] M. Cygler, P. Grochulski, R.J. Kazlauskas, J.D. Schrag, F. Bouthillier, B. Rubin, A.N. Serreqi and A.K. Gupta, J. Am. Chem. Soc., 116 (1994) 3180–3186.
[6] E. Rogalska, C. Cudrey, F. Ferrate and R. Verger, Chirality, 5 (1993) 24–30.
[7] P. Stadler, A. Kovac, L. Haalck, F. Spener and F. Paltauf, Eur. J. Biochem., 227 (1995) 335–343.
[8] A.N.E. Weissfloch and R.J. Kazlauskas, J. Org. Chem., 60 (1995) 6959–6965.
[9] M. Norin, F. Haeffner, A. Achour, T. Norin and K. Hult, Protein Sci., 3 (9) (1994) 1493–1503.
[10] D.M. Lawson, A.M. Brzozowski, S. Rety, C. Vemag and G.G. Dodson, Protein Eng., 7 (1994) 543–550.
[11] B. Vasel, H.J. Hecht, R.D. Schmid and D. Schomburg, J. Biotechnol., 28 (1993) 99–105.
[12] H.-D. Beer, G. Wohlfarth, J.E.G. McCarthy, D. Schomburg and R.D. Schmid, Protein Eng., 9 (1996) 507–517.
[13] M. Clark, R.D. Cramer III and N. van Opdenbosch, J. Comp. Chem., 10 (8) (1989) 982–1012.
[14] J.J.P. Stewart, J. Comp. Chem., 10 (1989) 209–220.
[15] H.J.C. Berendsen, J.P.M. Postma and W.F. van Gunsteren, J. Chem. Phys., 81 (8) (1984) 3684–3690.
[16] P.J. Goodford, J. Med. Chem., 28 (1985) 849–857.
[17] U. Derewenda, A.M. Brzozowski, D.M. Lawson and Z.S. Derewenda, Biochemistry, 31 (1992) 1532–1541.
[18] L. Swenson, R. Green, R. Joerger, M. Haas, K. Scott, Y. Wei, U. Derewenda, D. Lawson and Z.S. Derewenda, Proteins, 18 (3) (1994) 301–306.
[19] U. Derewenda, L. Swenson, Y. Wei, R. Green, P.M. Kobos, R. Joerger, M.J. Haas and Z.S. Derewenda, J. Lipid Res., 35 (3) (1994) 524–534.
[20] G. Zandonella, L. Haalck, F. Spener, K. Faber, F. Paltauf and A. Hermetter, Eur. J. Biochem., 231 (1995) 50–55.
[21] J. Broos, A.J.W.G. Visser, J.F.J. Engbersen, W. Verboom, A. van Hoeck and D.N. Reinhoudt, J. Am. Chem. Soc., 117 (51) (1995) 12657–12663.
[22] G. Wipff, A. Dearing, P.K. Weiner, J.M. Blaney and P.A. Kollman, J. Am. Chem. Soc., 105 (1983) 997–1005.
[23] J. Uppenberg, N. Oehrner, M. Norin, K. Hult, G.J. Kleywegt, S. Patkar, V. Waagen, T. Anthonsen and T.A. Jones, Biochemistry, 34 (1995) 16838–16851.
[24] P. Grochulski, F. Bouthillier, R.J. Kazlauskas, A.N. Serregi, J.D. Schrag, E. Ziomek and M. Cygler, Biochemistry, 33 (1994) 3494–3500.
[25] M.P. Egloff, F. Marguet, G. Buono, R. Verger, C. Cambillau and H. van Tilbeurgh, Biochemistry, 34 (1995) 2751–2762.
[26] E. Rogalska, S. Ransac and R. Verger, J. Biol. Chem., 256 (1990) 20271–20276.
[27] A. Kovac, P. Stadler, L. Haalck, F. Spener and F. Paltauf, Biochim. Biophys. Acta, 1301 (1996) 57–66.
[28] R.D. Joerger and M.J. Haas, Lipids, 29 (6) (1994) 377–384.