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# Computer-aided molecular modeling of the enantioselectivity of *Pseudomonas cepacia* lipase toward γ- and δ-lactones

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**Abstract**

Computer-aided molecular modeling was performed to investigate the experimentally determined enantioselectivities of *Pseudomonas cepacia* lipase (PCL) toward various saturated γ- and δ-lactones. Experimental data indicated that PCL preferentially hydrolyzes the (*R*)-enantiomers of both types of substrates. Interactions between the non-polar aliphatic alkyl chain of the (*S*)-enantiomers and the polar side chain of residue Y29 were identified to mediate enantioselectivity. Upon binding, the tyrosine was displaced, thus initiating a cascade of local geometry changes which led to the breakdown of the essential H-bond network at the active site H286. The lactone ring of the (*S*)-δ-enantiomers further added to this process, since it was forced into an unfavorable position by repulsion from Y29, directly affecting the position of H286. In contrast, the respective (*R*)-enantiomers fit without distorting side chains essential for catalysis in the binding pocket of PCL. In δ-lactones, the stereocenter was located close to the imidazole ring of H286, suggesting a more intense interaction with H286 as compared to γ-lactones. The length of the aliphatic chain adjacent to the stereocenter also affected the enantiopreference toward hydrolysis of δ-lactones, while for γ-lactones, the enantioselectivity did not significantly change with increasing alkyl chain length. In the cases of (*S*)-δ-octa- and (*S*)-δ-nonalactone, two alternative possible binding modes were examined, indicating that the respective substrate resolutions led to poor enantioselectivity as compared to the longer-chain δ-lactone substrates. 

*Keywords:* Molecular modeling; *Pseudomonas cepacia* lipase; Enantioselectivity; γ-Lactone; δ-Lactone

## 1. Introduction

Lactones are important intermediates both in organic synthesis and flavor compounds. As for many flavor and fragrance substances, in some lactones, a stereocenter is found generating enantiomers with different olfactory properties [1]. Different enantiomeric compositions of saturated lactones with five- and six-membered rings (γ- and δ-lactones, respectively) occur in cheddar cheese, raspberries, peaches, coconuts, osmanthus oil, and dairy products [2]. The lipase-catalyzed hydrolysis of such lactones has

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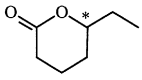
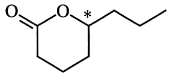
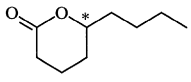
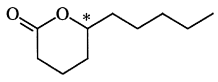
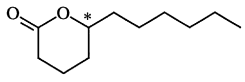
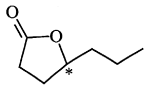
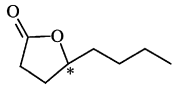
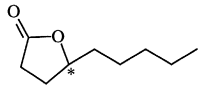
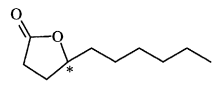
been thoroughly studied [3–5]. The most prominent results were obtained for Porcine pancreatic lipase and the *Pseudomonas cepacia* lipase (PCL). Enzelberger et al. [3] studied the enan-

Table 1
Structures of (*R*)- and (*S*)-δ-lactones, geometry, and experimentally determined *E*-values
Sterical and electrostatic interactions between the substrate and PCL can be monitored by measuring the distances of the $N_{\varepsilon}2$ of the imidazole ring in the active site H286 to $O_{\gamma}$ of the active site S87 ($N_{\varepsilon}$-$O_{\gamma}$), to O1 of the lactone ring ($N_{\varepsilon}$-O), and to $C_{\gamma}$ of active site D264 ($N_{\varepsilon}$-$C_{\gamma}$), respectively. The tolerance for the displacement of H286 imidazole without breaking down the H-bond network was defined to a $(R)_{His}$ and $(S)_{His}$ value $\leq 0.10$ Å, respectively. For the (*R*)-enantiomers, the H286 side chain remained nearly unaffected, while for most (*S*)-enantiomers, the imidazole ring was moved out of its favored position. (*S*1)-δ-octalactone and (*S*2)-δ-nonalactone did not significantly influence the position of H286 imidazole.

| Substrate | Enantiomer | $N_{\varepsilon}$-$O_{\gamma}$ | $N_{\varepsilon}$-O | $N_{\varepsilon}$-$C_{\gamma}$ | $(R)_{His}$ $(S)_{His}$ | E [1)] |
|---|---|---|---|---|---|---|
| δ-octalactone | (R) | 3.2 Å | 2.6 Å | 5.5 Å | 0.06 Å | 2 |
|  | (S1) | 3.3 Å | 2.5 Å | 5.4 Å | 0.08 Å | |
| | (S2) | 3.0 Å | 2.9 Å | 5.0 Å | **0.31 Å** | |
| δ-nonalactone | (R) | 3.2 Å | 2.6 Å | 5.4 Å | 0.00 Å | 4 |
|  | (S1) | 3.2 Å | 3.0 Å | 5.0 Å | **0.33 Å** | |
| | (S2) | 3.3 Å | 2.5 Å | 5.4 Å | 0.08 Å | |
| δ-decalactone | (R) | 3.2 Å | 2.7 Å | 5.4 Å | 0.06 Å | 45 |
|  | (S1) | 4.4 Å | 4.4 Å | 4.1 Å | **1.46 Å** | |
| | (S2) | 3.0 Å | 3.0 Å | 5.6 Å | **0.28 Å** | |
| δ-undecalactone | (R) | 3.3 Å | 2.6 Å | 5.5 Å | 0.08 Å | >100 |
|  | (S1) | 3.0 Å | 2.8 Å | 5.0 Å | **0.28 Å** | |
| | (S2) | 3.0 Å | 2.8 Å | 5.0 Å | **0.28 Å** | |
| δ-dodecalactone | (R) | 3.3 Å | 2.7 Å | 5.4 Å | 0.08 Å | >100 |
|  | (S1) | 3.5 Å | 2.4 Å | 5.6 Å | **0.24 Å** | |
| | (S2) | 3.0 Å | 2.8 Å | 5.0 Å | **0.28 Å** | |

[1)] Experimentally determined *E*-values from Ref. [3].

Table 2
Structures of (*R*)- and (*S*)-γ-lactones, geometry, and experimentally determined *E*-values
Sterical and electrostatic interactions between the substrate and PCL can be monitored by measuring the distances of the $N_\varepsilon$ of the imidazole ring in the active site H286 to $O_\gamma$ of the active site S87 ($N_\varepsilon$-$O_\gamma$), to O of the lactone ring ($N_\varepsilon$-O), and to $C_\gamma$ of active site D264 ($N_\varepsilon$-$C_\gamma$), respectively. The tolerance for the displacement of H286 imidazole without breaking down the H-bond network was defined to a $(R)_{His}$ and $(S)_{His}$ value $\leq 0.10$ Å, respectively. For both the (*R*)- and (*S*)-enantiomers, the position of H286 remained nearly unaffected.

| Substrate | Enantiomer | $N_\varepsilon$-$O_\gamma$ | $N_\varepsilon$-O | $N_\varepsilon$-$C_\gamma$ | $(R)_{His}$ $(S)_{His}$ | E [1)] |
|---|---|---|---|---|---|---|
| γ-octalactone | (R) | 3.3 Å | 2.6 Å | 5.5 Å | 0.08 Å | 4 |
|  | (S) | 3.0 Å | 2.8 Å | 5.5 Å | **0.17 Å** | |
| γ-nonalactone | (R) | 3.1 Å | 2.7 Å | 5.4 Å | 0.08 Å | 11 |
|  | (S) | 3.1 Å | 2.9 Å | 5.5 Å | **0.19 Å** | |
| γ-decalactone | (R) | 3.3 Å | 2.6 Å | 5.4 Å | 0.06 Å | 9 |
|  | (S) | 3.2 Å | 2.7 Å | 5.6 Å | **0.13 Å** | |
| γ-undecalactone | (R) | 3.2 Å | 2.7 Å | 5.5 Å | 0.08 Å | 8 |
|  | (S) | 3.4 Å | 2.4 Å | 5.5 Å | **0.17 Å** | |

[1)] Experimentally determined *E*-values from Ref. [3].

tioselectivity of PCL toward the hydrolysis of γ- and δ-lactones with aliphatic alkyl chains attached to the lactone ring. In case of the δ-lactones (δ-octa-, δ-nona-, δ-deca-, δ-undeca-, and δ-dodecalactone), the enantioselectivity of PCL toward the short-chained δ-octa- and δ-nona-compounds was poor, while longer alkyl chains significantly increased the enantioselectivity (Table 1). For the γ-lactones, the enantioselectivity was found to be poor for all substrates and, in most cases, independent from the alkyl chain length (Table 2).

Computer-aided molecular modeling has successfully been applied to investigate the enantioselectivity of *Mucorales* lipases toward triacylglycerols and analogs [6,7]. In our work, we extended this modeling procedure to a different class of substrates in order to identify the molecular reasons and to understand the enantioselectivity of PCL toward saturated γ- and δ-lactones.

## 2. Methods

The X-ray structure of the open conformation of PCL (PDB entry 2lip [8]) was obtained from

the Protein Data Bank (PDB) [9]. Structural models of the substrates were created using SYBYL 6.4 (Tripos, St. Louis, MO) and docked into the binding site mimicking the first tetrahedral intermediate which is the rate-limiting step of ester hydrolysis in lipases. The charge distribution at the catalytic residues S87, H286, and the substrate was modified as calculated by the semi-empirical method, MNDO94/PM3 [10], according to Holzwarth et al. [11].

For the (*S*)-δ-lactones, two initial structures, (*S*1) and (*S*2), were investigated. With the respective (*R*)-enantiomer as a template, the docking of the (*S*1)-δ-lactones was guided by the position of both the ring and the alkyl chain of the (*R*)-enantiomers. Due to the different configuration at the stereocenter of the lactone ring in (*R*)- and (*S*)-forms, the alkyl chains in the (*S*)-enantiomers were located closer to the residues T18 and Y29 as compared to the (*R*)-enantiomers. In the (*S*2)-δ-lactone complexes, the substrates were more favorably docked into the binding site of PCL. This was achieved by twisting the lactone ring toward the active site H286, revealing reduced repulsion of the alkyl chain with the lipase in the starting structure.

The initial PCL–substrate complexes were refined in vacuo by energy minimization and subsequent molecular dynamics simulations [6]. We applied position constraints to the enzyme backbone. Both side chains and substrate were allowed to move. The system was heated in three intervals of 2 ps each at 5, 30, and 70 K and 10 ps at 100 K, followed by a production phase of 2 ps at 100 K. The step size was set to 1 fs for the first two heating steps (5 and 30 K) and to 0.5 fs for the latter two (70 and 100 K) as well as the production phase (100 K). The non-bonded interaction cutoff was 8 Å, the coupling constant 10 fs, and the dielectric constant $\varepsilon = 1$, respectively [6]. An average structure was created by superimposing and averaging the 50 PCL–substrate complex structures of the production interval. The preferably hydrolyzed enantiomers were identified analyzing the distance of the $N_{\varepsilon}2$ in the imidazole ring of the active site H286 to $O_{\gamma}$ of the active site S87, $C_{\gamma}$ of the active site D264, and O1 of the substrate. These distances could be summarized to a characteristic $(R)_{\mathrm{His}}$ and $(S)_{\mathrm{His}}$ value, respectively. $(R)_{\mathrm{His}}$ and $(S)_{\mathrm{His}}$ are calculated from the following formula:

$$(R)_{\mathrm{His}}/(S)_{\mathrm{His}} = \sqrt{\frac{1}{3}\left(\left(X_{\mathrm{N}\varepsilon\text{-}\mathrm{O}\gamma} - 3.2\right)^2 + \left(X_{\mathrm{N}\varepsilon\text{-}\mathrm{O}} - 2.6\right)^2 + \left(X_{\mathrm{N}\varepsilon\text{-}\mathrm{C}\gamma} - 5.4\right)^2\right)}$$

These values give a single measure at hand for the displacement of the H286 imidazole ring in the PCL binding site. For each distance measured, a tolerance value of $\pm 0.1$ Å was introduced. The tolerance was derived from the respective distances in all investigated (*R*)-enantiomers. A tolerance value of 0.1 Å and less indicated only insignificant displacement of H286 imidazole. The catalytic machinery of PCL was, therefore, not destroyed. A tolerance value of more than 0.1 Å indicated strong H286 displacement and the breakdown of the essential H-bonding network.

## 3. Results

### 3.1. δ-Lactones

All (*R*)-enantiomers (δ-octa-, δ-nona-, δ-deca-, δ-undeca-, and δ-dodecalactone, respectively) were oriented into the substrate binding site with the aliphatic alkyl chain bound to the cavity (Fig. 1) which is formed by five non-polar (V26, L27, F146, I290, and L293) and five polar residues (T18, Y29, P243, S244, and Q292). Repulsive interactions, both sterical and

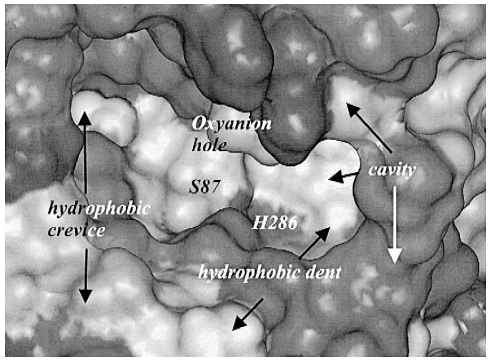



Fig. 1. The substrate binding site of PCL in Connolly representation. The S87 and H286 residues of the catalytic triad (S87, D264, H286) are labeled as well as the oxyanion hole (L17, Q88), the *hydrophobic crevice* (L17, P113, S117, F119, V123, L164, L167, and V267), the *hydrophobic dent* (L248, T251, V266, and L287), and the *cavity* (T18, V26, L27, Y29, F146, P243, S244, I290, Q292, and L293).

electrostatic, between the substrate and the lipase were not observed. Hydrogen bonds indicating productive ester hydrolysis were formed between the backbone of L17 and Q88 (oxyanion hole; Fig. 1) and the oxyanion, between $N_{\varepsilon}2$ of the catalytic H286 imidazole and $O_{\gamma}$ of S87 as well as O1 of the substrate. The formation of these essential hydrogen bonds was due to the correct placement of H286 imidazolee which could be monitored by measuring the distance between $N_{\varepsilon}2$ of H286 and $O_{\gamma}$ of the active site S87, $C_{\gamma}$ of the catalytically active D264, and O1 of the lactone ring. The distance of the H286 imidazole ring in an active PCL–substrate complex was determined to be 3.2 Å (with a tolerance of $\pm$0.1 Å) for $N_{\varepsilon}$ (H286)-$O_{\gamma}$ (S87), 2.6 Å ($\pm$0.1 Å) for $N_{\varepsilon}$ (H286)-O1 (substrate), and 5.4 Å ($\pm$0.1 Å) for $N_{\varepsilon}$ (H286)-$C_{\gamma}$ (D264) (Table 1). Calculating a rms deviation from these distances, $(R)_{His}$ and $(S)_{His}$, respectively, the relative position of H286 imidazole could be determined (Table 1). For the respective PCL-δ-lactone complexes, a $(R)_{His}$ and $(S)_{His}$ value of 0.10 Å was found to be tolerated by the system, while larger values indicated a breakdown of, at least, parts of the essential H-bonds. The $(R)_{His}$ values for all (*R*)-δ-lactones were in the range of 0–0.08 Å (Table 1).

For (*S*)-δ-lactones, sterical hindrance and electrostatic repulsion occurred between the alkyl chain of the lactone and residues in the binding site of the lipase. The (*S*1)-δ-lactone complexes, which were docked analogous to the (*R*)-enantiomers previous to energy minimization, were, during the course of molecular dynamics simulations, either oriented into the cavity (δ-octa- and δ-undecalactone) or the *hydrophobic dent* (δ-nona-, δ-deca-, and δ-dodecalactone). The latter consists of residues L248, T251, V266, and L287. The alkyl chains of the substrates interacted with residues of the cavity (T18, Y29, F146, I290, Q292, and L293) or of the *hydrophobic dent* (L287). During the course of molecular dynamics simulations, the side chain of H286 was moved out of its favorable position in an active lipase–substrate complex, except for (*S*1)-δ-octalactone (Fig. 2). The tolerable $(S)_{His}$ value of 0.10 Å was significantly

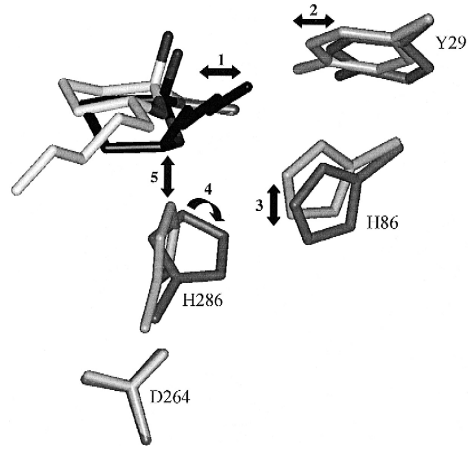



Fig. 2. The superimposed average structures of (*R*)-δ- (gray) and (*S*)-δ-undecalactone (black) complexes. The residues of the (*R*)-enantiomer are shaded in bright; residues of the (*S*)-enantiomers in dark gray. The non-polar alkyl chain of the (*S*)-enantiomer interacted with the polar side chain of Y29 (1). Y29 was displaced (2) with the polar hydroxy group pointing toward H86. This change in local geometry and electrostatics influenced the position of H86 (3) which is the C-terminal neighbor of the active site S87 and was postulated to stabilize the position of the active site H286. Hence, the side chain of H286 was no longer stabilized from H86 and shifted out of its favorable position (4). Additionally, interactions between the alkyl chain and Y29 affected the position of the stereocenter in the lactone ring which was pushed toward the imidazole ring of H286 (5).

exceeded. The $(S)_{His}$ values for (*S*1)-δ-nona-, (*S*1)-δ-deca-, (*S*1)-δ-undeca-, and (*S*1)-δ-dodecalactone were determined to be 0.33, 1.46, 0.28, and 0.24 Å, respectively (Table 1). In case of (*S*1)-δ-octa- and δ-undecalactone, Y29 was forced toward the core of the protein. The polar hydroxy group was pushed away from the non-polar substrate chain toward H86 which was also displaced (Fig. 2). A special case, the position of H286 in the PCL-(*S*1)-δ-octalactone complex, remained nearly unaffected ($(S)_{His}$ value 0.08 Å), but the neighboring H86, which supposedly stabilizes H286, was shifted. For the complexes hosting (*S*1)-δ-nona-, δ-deca-, and δ-dodecalactone, respectively, the alkyl chains were placed into the *hydrophobic dent* with Y29 not being vigorously influenced by the substrate. The hydroxy group pointed toward the substrate.

The (*S*2)-δ-lactones, which were modeled from a different starting structure, always hosted the lactone alkyl chains in the *cavity*. Sterical and electrostatic interactions between the substrate and mainly Y29 of PCL forced the tyrosine to displace its hydroxy group toward H86, which was also moved out of its position as was H286 (Table 1). Additionally, the lactone rings of the substrates bumped against the catalytic H286 enhancing the displacement of the imidazole ring. The $(S)_{His}$ values were 0.31 Å for (*S*2)-δ-octalactone and 0.28 Å for (*S*2)-δ-deca-, (*S*2)-δ-undeca-, (*S*2)-δ-dodecalactone, respectively. For (*S*2)-δ-nonalactone, H286 was hardly influenced by the substrate. The corresponding $(S)_{His}$ value was determined to be 0.08 Å (Table 1).

### 3.2. γ-Lactones

For the five-membered γ-lactones, both (*R*)- and (*S*)-enantiomers oriented their alkyl chains toward the *hydrophobic dent*, except for the (*R*)-γ-undecalactone which bound its aliphatic tail into the *cavity*. The substrates hardly interacted with the lipase. Interactions only occurred sterically with L287. The longer the aliphatic tail, the more intense the sterical interaction with L287. The position of H286 was barely affected by the substrates (Table 2, Fig. 3). For (*R*)-γ-undecalactone, the alkyl chain directed more toward the *hydrophobic dent* as compared to the respective δ-lactones avoiding repulsion with the lipase.

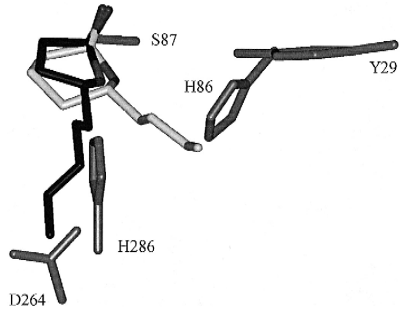



Fig. 3. The superimposed average structures of (*R*)-δ- (gray) and (*R*)-γ-decalactone (black) complex. The residues of the catalytic triad (S87, D264, and H286) are displayed as well as H86 which supposedly stabilizes the position of H286. Y29 exhibited the most intense interaction with the substrates.

As for the δ-lactones, the maximum $(R)_{His}$ and $(S)_{His}$ value, respectively, for an active PCL–substrate complex was found to be 0.10 Å with the catalytically relevant H-bonds from H286 to S87 and the substrate remaining almost unaffected. The relative position of H286 toward S87, D264, and the lactone ring was equal to the results for δ-lactones: $3.2 \pm 0.1$ Å for $N_\varepsilon$ (H286)-$O_\gamma$ (S87), $2.6 \pm 0.1$ Å for $N_\varepsilon$ (H286)-O (substrate), and $5.4 \pm 0.1$ Å for $N_\varepsilon$ (H286)-$C_\gamma$ (D264). In case of (*R*)-γ-lactones, the $(R)_{His}$ values were calculated to be 0.06 Å ((*R*)-γ-decalactone) and 0.08 Å ((*R*)-γ-octa-, (*R*)-γ-nona-, and (*R*)-γ-undecalactone), respectively. For (*S*)-γ-lactones, the $(S)_{His}$ values were determined to be 0.17 Å ((*S*)-γ-octalactone), 0.19 Å ((*S*)-γ-nonalactone), 0.13 Å ((*S*)-γ-decalactone), and 0.17 Å ((*S*)-γ-undecalactone), respectively (Table 2).

## 4. Discussion

In this work, we performed molecular modeling studies using the X-ray structure of the open form of PCL and compared these data to the

experiments of Enzelberger et al. [3] using *Pseudomonas* species lipase (KW 51) (PSpL). Comparing sequences of PCL [12] and PSpL [13,14] exhibited that both lipases are homologous, with 93% identical and an additional 6% similar residues. Therefore, the data generated from molecular modeling were reasonable to be compared to the experimental work.

The substrate binding site of PCL consists of three putative, mostly hydrophobic binding regions for substrates: the *hydrophobic crevice* always hosts the hydrolyzed acid residue, while the *hydrophobic dent* and the *cavity* both can bind the alcohol residue (Fig. 1). Previously, from the X-ray structure of PCL, Schrag et al. [8] identified a *boomerang-shaped* cleft surrounding the active site and, therefore, embodying the substrate binding region. This cleft was made up from residues 17–29 and from three helices consisting of residues 118–127, 134–150, and 243–256 which limited its size. However, we identified the residues L287, I290, L293, and Q292 to also participate in the formation of the alcohol binding pocket lying on a 22-residue loop.

The modeling procedure was originally developed for complexes of *Mucorales* lipases with triacylglycerol substrates and analogs [6,7]. In this work, this procedure was further broadened to a different class of substrates: γ- and δ-lactone substrates hydrolyzed by PCL. Enzelberger et al. [3] evaluated that the homologous PSpL preferably hydrolyzed the (*R*)-enantiomers of both γ- and δ-lactones. Molecular modeling indicated that the enantiopreference of PCL toward the (*R*)- rather than the (*S*)-enantiomers is most likely due to sterical and electrostatic interactions between the alkyl substituent of the (*S*)-enantiomers and residue Y29 which were not observed for the (*R*)-enantiomers. The intensity of these interactions varied for γ- and δ-lactones as well as for lactone substrates with different chain length.

The most intense substrate–lipase interactions occurred for δ-lactone complexes. For (*S*1)-δ-octa-, (*S*1)-δ-undeca-, and all (*S*2)-δ-lactones, the alkyl chains of the substrates forced the polar hydroxy group of the tyrosine to move away from the substrate initiating a *cascade* (Fig. 2). The hydroxy group of Y29, eventually, pointed toward H86, which is the C-terminal neighbor of the catalytically active S87. The respective residue H144 in *Rhizopus* lipase was supposed to stabilize the position of the active site, histidine [15]. Therefore, the displacement of the H86 side chain resulted in a breakdown of the stabilizing effect of H86 on H286 in PCL. The consequent shift of the H286 imidazole could be easily monitored by measuring its relative position and, subsequently, calculating the respective $(R)_{His}$ and $(S)_{His}$ values for the PCL complexes with (*R*)- and (*S*)-enantiomers as a measure for the displacement of the H286 imidazole ring toward the unaffected $O_\gamma$ of S87, $C_\gamma$ of D264, and O1 of the lactone ring. For (*S*1)-δ-nona-, (*S*1)-δ-deca-, and (*S*1)-δ-dodecalactone, this cascade of local geometry changes did not occur, since during the course of molecular dynamics simulations, the aliphatic alkyl chains were moved to the *hydrophobic dent* rather than the *cavity*. This displacement resulted in an unfavorable kink in the otherwise all-*trans*-oriented aliphatic alkyl tail. However, the relative position of the active site H286 was influenced as in the (*S*)-δ-enantiomers which were bound to the *cavity*.

Further outcome on the position of H286 in PCL complexes with δ-lactones resulted from the configuration at the stereocenter of the lactone ring. In (*S*)-enantiomers, interactions between the substrate and Y29 also directly affected the orientation of the lactone ring which was pushed toward the active site, H286. This boosted the destabilization of the side chain. The different starting structures, (*S*1) and (*S*2), were not found to notably influence the results generated from molecular modeling studies. All (*S*)-δ-enantiomers affected the position of H286 indicated by the values for $(S)_{His}$ value (Table 1).

Enzelberger et al. [3] already discussed these repulsions as consequences of the ring size and

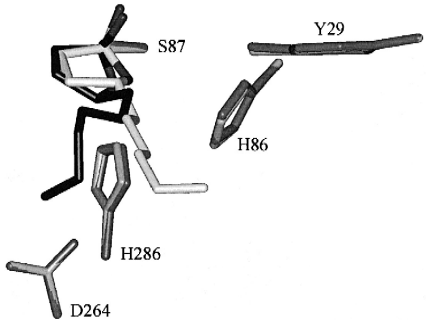



Fig. 4. The superimposed average structures of (*R*)-γ- (gray) and (*S*)-γ-undecalactone (black) complexes. The residues of the (*R*)-enantiomer are shaded in bright; residues of the (*S*)-enantiomers in dark gray. Both enantiomers properly fit into the binding pocket.

the more rigid conformation of the six-membered ring in δ-lactones. Our results support this theory, since in δ-lactone complexes, the ring is located in direct contact to H286 as compared to the more flexible five-membered ring in γ-lactones (Figs. 3 and 4). Experimental studies revealed that the enantiopreference of PCL toward δ-lactones increased from poor *E*-values of two and four toward δ-octa-and δ-nonalactone, respectively, to moderate $E = 45$ toward δ-decalactone, and high values of $E > 100$ toward δ-undeca-, and δ-dodecalactone, respectively (Table 1), while toward the γ-lactones, the enantioselectivity of PCL did not significantly change with the chain length of the aliphatic alkyl chain (Table 2).

The short-chained δ-octa- and δ-nonalactone substrates were able to occupy alternative positions in the binding site of PCL. The (*S*1)-δ-octalactone enantiomer, which was bound similarly to the respective (*R*)-enantiomer, and the (*S*2)-δ-nonalactone enantiomer could, therefore, bind into the substrate binding site such that the position of the catalytically active H286 remained unaffected (Table 1). Hence, both alternative positions revealed only weak interactions between the substrate and the lipase which may explain the poor enantioselectivities of PCL toward these substrates as compared to the notably high values toward the longer-chained δ-lactones. In case of the γ-lactone complexes, the alkyl chain length did not correlate to enantioselectivity, since the interactions between substrates and PCL were weak for both (*R*)- and (*S*)-enantiomers (Table 1).

Fig. 5 summarizes these observations. A correlation between the displacement of H286 and the experimentally determined *E*-value could be found. A $\Delta(S)_{\mathrm{His}} - (R)_{\mathrm{His}}$ value (difference between $(S)_{\mathrm{His}}$ and $(R)_{\mathrm{His}}$) could be calculated leading to a comparable measure independent of molecular modeling results for a given PCL–substrate complex. For δ-lactones, from (*S*1)- and (*S*2)-enantiomers, the smaller $\Delta(S)_{\mathrm{His}} - (R)_{\mathrm{His}}$ values and, thus, the more favorably oriented (*S*)-enantiomers were selected. Clustering was observed for the hydrolysis of γ- and δ-lactone substrates with poor, moderate or good enantioselectivities. A value of $\Delta(S)_{\mathrm{His}} - (R)_{\mathrm{His}} < 0.12$ Å correlated to poor, of $\Delta(S)_{\mathrm{His}} - (R)_{\mathrm{His}} > 0.15$ Å to moderate or good enantioselectivities. Therefore, our procedure is adequate to semi-quantitatively predict the enantioselectivity of γ- and δ-lactone hydrolysis by PCL.

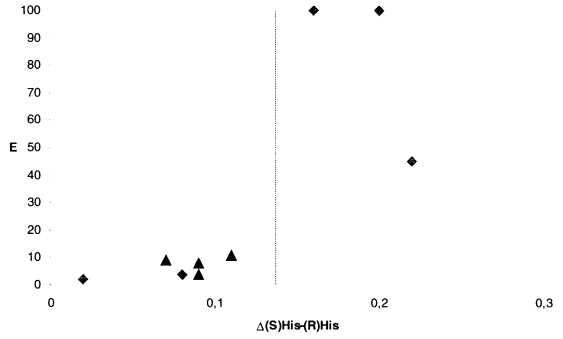



Fig. 5. Correlation diagram between experimentally determined *E* and $\Delta(S)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$ values. The $\Delta(S)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$ values were calculated as the difference between the $(S)_{\mathrm{His}}$ values of (*S*)- and the respective $(R)_{\mathrm{His}}$ values of the (*R*)-enantiomers. In case of δ-lactones, the smaller $\Delta(S)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$ value was selected (either $\Delta(S1)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$ or $\Delta(S2)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$). For δ-octa- and δ-dodecalactone, the $\Delta(S1)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$ value, for δ-nona- and δ-decalactone, the $\Delta(S2)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$ value was picked, while for δ-undecalactone, both $\Delta(S)_{\mathrm{His}}$-$(R)_{\mathrm{His}}$ values were equal. δ-Lactones are highlighted as (◆), γ-lactones as (▲). The dotted line discriminates between substrates which are hydrolyzed with moderate to good (right) and poor (left) enantioselectivities.

## 5. Conclusion

Molecular modeling was used to identify and explain the different enantioselectivities of PCL toward γ- and δ-lactones with aliphatic alkyl chains of different chain length adjacent to the stereocenter. Displacement of side chains caused by sterical and electrostatic repulsions correlates to enantioselectivity. Enantioselectivity toward lactones increased due to intense lipase–substrate interactions. For (*R*)-δ-, (*R*)-γ-, and (*S*)-γ-lactones, interactions between PCL and the substrates were weak, while for the (*S*)-δ-enantiomers, sterical and electrostatic repulsions between the lactone ring and the imidazole ring of H286 as well as between the alkyl chain and Y29 affected the position of the active site H286. For the short-chained (*S*)-δ-octa- and δ-nonalactones, alternative substrate orientations exist in the binding site of PCL which cannot be occupied by long-chained substrates, thus, reducing repulsion and, therefore, enantioselectivity.

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