A Three-Dimensional Predictive Active Site Model for Lipase from *Pseudomonas cepacia*

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A three-dimensional active site model of lipase from *Pseudomonas cepacia*—one of the most popular lipases in organic synthesis—was developed on the basis of the kinetic resolution of 3-(aryloxy)propan-2-ols. Size and shape of both hydrophobic binding pockets of the active site of this lipase were determined by substrate mapping in combination with molecular modeling for substrates and nonsubstrates. This model explains and predicts whether a compound is accepted as a substrate or not and allows to assess the enantiomer selectivity of the lipase-catalyzed reaction.

Introduction

The use of enzymes has been established as an important tool in organic synthesis during the past decade. Due to their ability to discriminate between enantiomers and enantiotopic groups, they are utilized in kinetic resolutions of racemates and asymmetrizations of prostereogenic or *meso* compounds to provide an easy access to enantiomerically pure building blocks, synthetic and natural products.[1]

Among the biocatalysts used in organic synthesis, lipases (triacylglycerol hydrolases, EC 3.1.1.3) have been used most frequently because they are cheap, available from many sources, easy to handle, and accept a broad range of substrates.[2] Furthermore, they are active in aqueous solution and in practically water-free organic solvents. Particularly, in organic solvents lipases remain their activity up to 100 °C. Lipases catalyze hydrolysis and formation of carboxylic esters and formation of amides upon the reaction conditions.

X-ray analyses of some lipases[3] evidence that their active sites are similar to those of serine proteases in which the primary hydroxy function of serine of the catalytic triade acts as a nucleophile to attack amide or ester carbonyl groups. Furthermore, these structure determinations show a common catalytic machinery for all lipases. Despite this fact, substrate acceptance and the degree of enantiodifferentiation is very different depending upon the natural source of the lipase. It is accepted in general that substrate recognition, stabilization, and enantioselective transformation is determined by two hydrophobic binding regions or pockets which are not separated from the catalytic site.[3f]

One of the most popular lipases used in organic synthesis is lipase from *Pseudomonas cepacia* from Amano Pharmaceutical Co., Ltd. (Nagoya, Japan), called lipase Amano PS. Before reidentification of the bacterial source it was called lipase from *P. fluorescens* (lipase P). Due to the flexibility of its binding sites lipase PS accepts a broad range of substrates. This lipase has been used for regio- and stereoselective hydrolysis[4] and alcoholysis[5] of carboxylic esters and anhydrides[6] and for the regio- and stereoselective transesterification of alcohols.[4a,b,d,7] There seems to be almost no restriction regarding the structure of compounds which are accepted as substrate by lipase PS.

In order to rationalize and to predict reactivity and selectivity of lipase-catalyzed biotransformations, a deeper

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(1) (a) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071. (b) Poppe, L; Novák, L. *Selective Biocatalysis*; VCH: Weinheim, 1992. (c) Roberts, S. M., Wiggins, K., Casy, G., Eds. *Preparative Biotransformations*; Wiley: Chichester, 1992. (d) Faber, K. *Biotransformations in Organic Chemistry*, 2nd ed.; Springer-Verlag: Heidelberg, 1995. (e) Drauz, K., Waldmann, H., Eds. *Enzyme Catalysis in Organic Synthesis*; VCH: Weinheim, 1995. (f) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.

(2) (a) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. (b) Boland, W.; Frössl, C.; Lorenz, M. *Synthesis* **1991**, 1049. (c) Faber, K; Riva, S. *Synthesis* **1992**, 895. (d) Santaniello, E.; Ferraboschi, P.; Grisenti, P. *Enzyme Microb. Technol.* **1993**, *15*, 367. (e) Theil, F. *Catal. Today* **1994**, *22*, 517. (f) Theil, F. *Chem. Rev.* **1995**, *95*, 2203. (g) Koskinen, A. M. P., Klibanov, A. M., Eds. *Enzymatic Reactions in Organic Media*; Blackie Academic & Professional: Glasgow, 1996.

(3) (a) Winkler, F. K.; D'Arcy, A.; Hunzicker, W. *Nature* **1990**, *343*, 771. (b) Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343*, 767. (c) Grochulski, P.; Li, Y.; Schrag, J. D.; Bouthillier, F.; Smith, P.; Harrison, D.; Rubin, B.; Cygler, M. *J. Biol. Chem.* **1993**, *268*, 12843. (d) Noble, M. E. M.; Cleasby, A.; Johnson, L. N.; Egmond, M. R.; Frenken, L. G. J. *FEBS Lett.* **1993**, *331*, 123. (e) Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. *Structure* **1994**, *2*, 293. (f) Cygler, M.; Grochulski, P.; Kazlauskas, R. J.; Schrag, J. D.; Bouthillier, F.; Rubin, B.; Serreqi, A. N.; Gupta, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 3180.

(4) (a) Klempier, N.; Faber, K.; Griengl, H. *Synthesis* **1989**, 933. (b) Foelsche, E.; Hickel, A.; Hönig, H.; Seufer-Wasserthal, P. *J. Org. Chem.* **1990**, *55*, 1749. (c) Goergens, U.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1064. (d) Goergens, U.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1066. (e) Chenevert, R.; Gagnon, R. *J. Org. Chem.* **1993**, *58*, 1054. (f) Hirose, Y.; Kariya, K.; Nakanishi, Y.; Kurono, Y.; Achiwa, K. *Tetrahedron Lett.* **1995**, *36*, 1063. (g) Tanaka, K.; Shogase, Y.; Osuga, H.; Suzuki, H.; Nakamura, K. *Tetrahedron Lett.* **1995**, *36*, 1675.

(5) (a) Bianchi, D.; Cesti, P. *J. Org. Chem.* **1990**, *55*, 5657. (b) Bianchi, D.; Bosetti, A.; Cesti, P.; Golini, P. *Tetrahedron Lett.* **1992**, *33*, 3231. (c) Koichi, Y.; Suginaka, K.; Yamamoto, Y. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1645.

(6) (a) Yamamoto, K.; Nishioka, T.; Oda, J.; Yamamoto, Y. *Tetrahedron Lett.* **1988**, *29*, 1717. (b) Ozegowski, R.; Kunath, A.; Schick, H. *Liebigs Ann. Chem.* **1993**, 805.

(7) (a) Kim, M.-J.; Choi, Y. K. *J. Org. Chem.* **1992**, *57*, 1605. (b) Moris, F.; Gotor, V. *J. Org. Chem.* **1992**, *57*, 2490. (c) Takano, S.; Yamane, T.; Takahashi, M.; Ogasawara, K. *Synlett* **1992**, 410. (d) Bovara, R.; Carrea, G.; Ottolina, G.; Riva, S. *Biotechnol. Lett.* **1993**, *15*, 169. (e) Bovara, R.; Carrea, G.; Ferrara, L.; Riva, S. *Tetrahedron: Asymmetry* **1991**, *2*, 931. (f) Lambusta, D.; Nicolosi, G.; Patti, A.; Piattelli, M. *Tetrahedron: Asymmetry* **1993**, *4*, 919. (g) Sato, M.; Hirokawa, T.; Hattori, H.; Toyota, A.; Kaneko, C. *Tetrahedron: Asymmetry* **1994**, *5*, 975. (h) Nakano, H.; Okuyama, Y.; Iwasa, K.; Hongo, H. *Tetrahedron: Asymmetry* **1994**, *5*, 1155. (i) Takano, S.; Yamada, O.; Iida, H.; Ogasawara, K. *Synthesis* **1994**, 592. (j) Sibi, M.-P.; Lu, J. L. *Tetrahedron Lett.* **1994**, *35*, 4915. (k) Boaz, N. W.; Zimmerman, R. L. *Tetrahedron: Asymmetry* **1994**, *5*, 153. (l) Chadha, A.; Manohar, M. *Tetrahedron: Asymmetry* **1995**, *6*, 651. (m) Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *Tetrahedron: Asymmetry* **1996**, *7*, 625. (n) Theil, F.; Ballschuh, S. *Tetrahedron: Asymmetry* **1996**, *7*, 3565.

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insight into the active sites of lipases regarding the catalytic mechanism as well as shape and size of the binding pockets is necessary. There are two approaches to contribute to the solution of this problem which are complementary one another: X-ray structure determination of crystalline lipases and substrate mapping.

Very recently, the structure of covalent complexes of *Candida rugosa* lipase with transition state analogs for the hydrolysis of menthyl esters was used to explain the chiral preference of lipases in general.[3f] However, lipases are typical induced-fit enzymes and therefore X-ray structure as a frozen conformation cannot be used to explain and predict which compound is accepted as a substrate by the lipase. In order to determine spatial and constitutional requirements to identify substrates and nonsubstrates substrate mapping in combination with molecular modeling seems to be very promising.

In order to rationalize substrate properties and to predict substrate properties for enzyme-catalyzed reactions, it is of great importance to develop active site models.

Cubic-space active site models have been developed for porcine liver esterase,[8] cyclohexanone monooxygenase for Baeyer–Villiger oxidation[9] or sulfoxidation,[10] a nitrilase,[11] and a very crude model for lipase YS from Amano.[12]

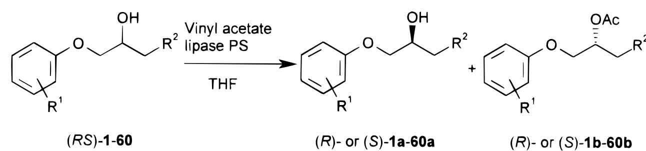
In the case of lipase from *P. cepacia*, there are some attempts to explain the enantiomer selectivity by different sizes of the hydrophobic pockets based on substrate mapping of this lipase without determining the shapes and sizes of the binding sites.[13] Kazlauskas' rules[14] explain and predict the stereochemical outcome for the kinetic resolution of esters by hydrolysis and transesterification of alcohols on the basis of different sizes of the substituents adjacent with the stereogenic center. A preliminary X-ray analysis of *P. cepacia* lipase[15] gives due to a low resolution no information on the active site of this enzyme.

Results and Discussion

It was our aim to develop a three-dimensional active site model of lipase from *P. cepacia* by combination of substrate mapping and molecular modeling.

Substrate Mapping. Very recently we found that reactivity and enantiomer selectivity in the kinetic

Scheme 1



resolution of 3-(aryloxy)propan-2-ol derivatives by transesterification with vinyl acetate in organic solvents in the presence of lipase from *P. cepacia* significantly depend on the substituents at the aromatic ring and in the 1-position of the propyl skeleton[16] (Scheme 1). In our concept probing the three-dimensional structure of the active site of lipase PS by substrate mapping, it was very important to identify nonsubstrates for this lipase. Nonsubstrates are compounds which differ from substrates in the size of the substituents at the stereogenic center and are not transformed in the presence of lipase from *P. cepacia*. The set of 61 3-(aryloxy)propan-2-ols screened as substrates can be subdivided regarding their chemical reactivity into substrates (Group 1) which are acylated with a different degree of enantiomer selectivity expressed as the E value[17] and nonsubstrates (Group 2) which resist lipase-PS-catalyzed transesterification as shown in Figure 1. With regard to their substitution pattern, compounds of Group 1 can be subdivided into the Subgroups 1.1–1.5 and those of Group 2 can be subdivided into the Subgroups 2.1 and 2.2 (Figure 1).

Summarizing the facts depicted in Figure 1, it can be concluded that reactivity and enantiomer selectivity of the lipase-catalyzed transesterification strongly depend on the substituents R^1 and R^2. In general, good substrates are characterized by R^1 representing hydrogen or *para* substituents at the aryl ring (compounds **1**–**8**, Subgroup 1.1 with $E > 50$) and by R^2 being an unbranched acyloxy residue (compounds **14**–**21**, Subgroup 1.2 with $E > 50$). Either short acyloxy groups such as acetate or extremely long acyloxy groups such as *n*-hexadecanoate are converted smoothly with high enantiomer selectivity. Branched residues such as isobutyrate **22**, 3-phenylpropanoate **23** and 6-phenylhexanoate **24** (Subgroup 1.2 with $E > 50$) are good substrates as well. Substrates in which R^1 is a small *ortho* substituent at the aryl ring (compounds **3**–**13**, Subgroup 1.1 with $E < 50$) and in which R^2 is a branched acyloxy (compounds **30**–**34**, Subgroup 1.2 with $E < 50$), an alkyloxy (compounds **35**–**38**, Subgroup 1.3), or azido (compound **39**, Subgroup 1.4) residue are converted with a significantly lower enantiomer selectivity. The amines **40** and **41** (Subgroup 1.4) are substrates for lipase PS, but in this case the enzyme is not able to distinguish between the enantiomers. However, chemical reaction could be excluded because in the absence of lipase PS the substrates **40** and **41** are not acylated by vinyl acetate. With the exception of the compound **48** substrates of Subgroup 1.5 are acylated by lipase PS with poor enantiomer selectivity.

When the isobutanoate **22** (Subgroup 1.2) and the pivaloate **56** (Subgroup 2.2) distinguished by a methyl group at the acyl residue in the 1-position are compared,

(8) (a) Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946. (b) Toone, E. J.; Jones, J. B. *Tetrahedron: Asymmetry* **1991**, *2*, 1041. (c) Provencher, L.; Wynn, H.; Jones, J. B.; Krawczyk, A. R. *Tetrahedron: Asymmetry* **1993**, *4*, 2025.

(9) Ottolina, G.; Carrea, G.; Colonna, S.; Rückmann, A. *Tetrahedron: Asymmetry* **1996**, *7*, 1123.

(10) Ottolina, G.; Pasta, P.; Carrea, G. Colonna, S.; Dallavalle, H.; Holland, L. *Tetrahedron: Asymmetry* **1995**, *6*, 1375.

(11) Deigner, H. P.; Blencowe, C.; Freyberg, C. E. *J. Mol. Catal. B* **1996**, *1*, 61.

(12) Naemura, K.; Fukuda, R.; Konishi, M.; Hirose, K.; Tobe, Y. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1253.

(13) (a) Laumen, K. Ph.D. Thesis, Universität-Gesamthochschule, Wuppertal, 1987. Kloosterman, M.; Kierkels, J. G. T.; Guit, R. P. M.; Vleugels, L. F. W.; Geladé, E. T. F.; van den Tweel, W. J. J.; Elferink; V. H. M.; Hulshof, L. A.; Kamphuis, J. In *Lipases: Structure, Mechanism and Genetic Engineering*; Alberghina, L., Schmid, R. D., Verger, R., Eds.; VCH: Weinheim, 1991; p 187. Xie, Z.-F. *Tetrahedron: Asymmetry* **1991**, *2*, 733. Exl, C.; Hönig, H.; Renner, G.; Rogi-Kohlenprath, R.; Seebauer, V.; Seufer-Wasserthal, P. *Tetrahedron: Asymmetry* **1992**, *3*, 1391.

(14) (a) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656. (b) Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 6959.

(15) Kim, K. K.; Hwang, K. Y.; Jeon, H. S.; Kim, S.; Sweet, R. M.; Yang, C. H. Suh, S. W. *J. Mol. Biol.* **1992**, *277*, 1258.

(16) (a) Theil, F.; Weidner, J.; Ballschuh, S.; Kunath, A.; Schick, H. *J. Org. Chem.* **1994**, *59*, 388. (b) Theil, F.; Lemke, K., Ballschuh, S.; Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1995**, *6*, 1323. (c) Lemke, K.; Theil, F. Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1996**, *7*, 971.

(17) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

Group 1 – Substrates

Dependence of enantiomer selectivity on R^1

Subgroup 1.1

Substrates **1–8**: R^2 = OAc (E > 50)

R^1 (E):	**1**: H (> 100)	**2**: 4-Me (> 100)	**3**: 2,4-Me_2 (80)
	4: 4-Cl (> 100)	**5**: 4-*t*-Bu (> 100)	**6**: 4-*t*-C_8H_{17} (> 100)
	7: 4-Ph (52)	**8**: 4-CO-Ph (> 100)	

Substrates **9–13**: R^2 = OAc (E < 50)

R^1 (E):	**9**: 2-Me (27)	**10**: 2-Cl (27)	**11**: 2,4-Cl (29)
	12: 4-CH_2Ph (15)	**13**: 4-OPh (26)	

Dependence of enantiomer selectivity on R^2

Subgroup 1.2

Substrates **14–24**: R^1 = H (E > 50)

unbranched acyl side chains

R^2 (E):	**14**: OCOMe (> 100)	**15**: OCOEt (52)	**16**: OCO*n*-Bu (54)
	17: OCO*n*-C_5H_{11} (> 100)	**18**: OCO*n*-C_7H_{15} (59)	**19**: OCO*n*-C_9H_{19} (> 100)
	20: OCO*n*-$C_{11}H_{23}$ (57)	**21**: OCO*n*-$C_{15}H_{31}$ (> 100)	

branched acyl side chains

	22: OCO*i*-Pr (78)	**23**: OCO(CH_2)$_2$Ph (> 100)	
	24: OCO(CH_2)$_5$Ph (> 100)		

Substrates **25–49**: R^1 = H (E < 50)

unbranched acyl side chains

R^2 (E)	**25**: OCO*n*-Pr (18)	**26**: OCO*n*-C_6H_{13} (25)	**27**: OCO*n*-C_8H_{17} (28)
	28: OCO*n*-$C_{13}H_{27}$ (25)	**29**: OCO*n*-$C_{17}H_{33}$ (31)	

branched acyl side chains

R^2 (E)	**30**: OCO*i*-Bu (16)	**31**: OCO*i*-C_5H_{11} (4)	**32**: OCO*cyclo*-C_6H_{11} (9)
	33: OCOPh (47)	**34**: OCOBn (9)	

Subgroup 1.3

Ethers

R^2 (E)	**35**: OMe (42)	**36**: OEt (37)	**37**: O*n*-Bu (15)
	38: O*i*-Pr (5)		

Subgroup 1.4

Nitrogen compounds

R^2 (E)	**39**: N_3 (17)	**40**: NEt_2 (1)	**41**: N*i*-Pr_2 (1)

Subgroup 1.5

R^1 = 4-*t*-C_8H_{17}

R^2 (E):	**42**: OCOEt (36)	**43**: OCO*n*-C_5H_{11} (36)	**44**: OCO*i*-C_5H_{11} (20)
	45: OCO*n*-C_9H_{19} (20)	**46**: OCO*n*-$C_{11}H_{23}$ (29)	**47**: OCO*n*-$C_{15}H_{31}$ (11)
	48: OCOPh (>100)	**49**: N_3 (29)	

Group 2 – Non-substrates

Subgroup 2.1

Non-substrates **50–55**: R^2 = OAc

R^1 =	**50**: 2-*t*-Bu	**51**: 2,4-di-*t*-Bu	**52**: 2-Ph
	53: 2-COPh	**55**: 2-CH_2Ph	

Subgroup 2.2

Non-substrates **56–61**: R^1 = H

R^2:	**56**: OCO*t*-Bu,	**57**: $OCPh_3$	**58**: OSi*t*-$BuMe_2$
	59: OSi*t*-$BuPh_2$	**60**: OSO_2Me	**61**: OSO_2*p*-Tol

Figure 1. Survey of 3-(aryloxy)propan-2-ol derivatives screened as substrates.

a significantly different behavior of both compounds was observed. While the isobutyrate **22** was smoothly converted by lipase PS with high enantiomer selectivity (E = 78), its homologue the pivaloate **56** completely resists lipase PS-catalyzed acylation. Based on this finding, it was predicted and found that comparable bulky substituents in the 1-position lead to nonsubstrates such as **57**–**61**. The nonsubstrates do not compete with substrates in accommodating the active site of lipase from *P. cepacia.* For example, if lipase PS is preincubated with the nonsubstrates **56**–**61** and then incubated with the isobutanoate **22**, the latter is acylated with the same rate and selectivity as in the absence of nonsubstrates. In general, the nonsubstrates **50**–**61** (Figure 2) are characterized either by a bulky substituent in the *ortho* position of the aromatic ring (Subgroup 2.1) or substituents in the 1-position in which the groups A and B are atoms with four ligands (all hydrogen replaced) (Subgroup 2.2).

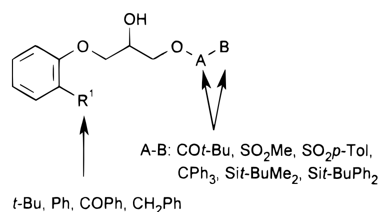


Figure 2. Structural requirements of nonsubstrates.

Scheme 2

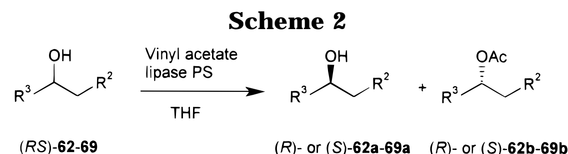


Table 1. Kinetic Resolution of the Ethanediol Derivatives (*RS*)-62–69

substrate	R^3	R^2	E
62	*n*-C_8H_{17}	OAc	4
63	*n*-$C_{10}H_{21}$	OAc	10
64	*n*-$C_{12}H_{25}$	OAc	8
65	*n*-$C_{14}H_{29}$	OAc	8
66	cyclo-C_6H_{11}O	OAc	>100
67	Ph	OAc	74
68	Ph	OCO-*n*-C_9H_{19}	47
69	Ph	OCO-*n*-$C_{15}H_{31}$	>100

In order to investigate the influence of the (aryloxy)methyl residue in general on reactivity and enantiomer selectivity, it was replaced by other substituents. Scheme 2 and Table 1 demonstrate the results for the kinetic resolution of ethane diol derivatives (*RS*)-**62**–**69** in which R^3 represents phenyl, *n*-alkyl, and cyclohexyloxy residues.

Table 1 demonstrates that the aryloxy substituent can be replaced by alkyl, phenyl, or the cyclohexyloxy residue. However, if R^3 is an *n*-alkyl chain as for the substrates **62**–**65**, the enantiomer selectivity drops down as has already been found for shorter alkyl groups.[16a] But very high selectivity was observed for the cyclohexyloxy derivative **66** which is evidence that the aromatic character of R^3 is not necessary for an efficient kinetic resolution. The phenyl ethanediol derivatives **67**–**69** show properties comparable to those found for the corresponding (aryloxy)methyl compounds **14**, **19**, and **21**. The poor enantiomer selectivity of the alkyl derivatives **62**–**65** compared with those compounds **66**, **67**, and the aryloxy derivatives of Group 1 show that a long alkyl side chain cannot mimic a cyclic residue by size and shape.

In general the enantiomer selectivity of the substrates acetylated by lipase PS is in accordance with Kazlauskas' rule. However, for substrates with long acyl or aromatic

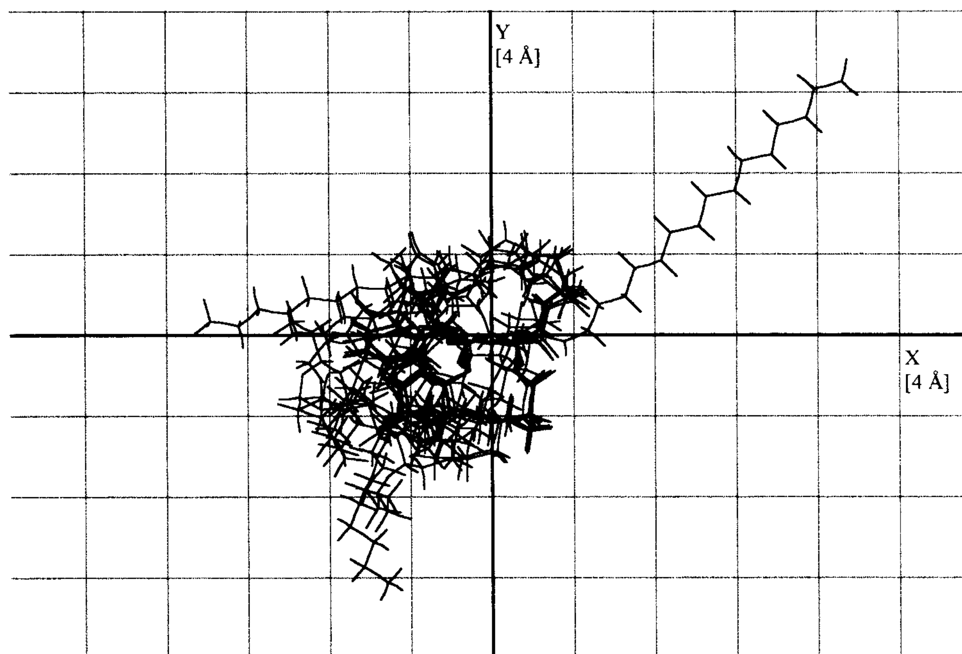


Figure 3. Superposition of 20 low-energy conformers of substrate **21**.

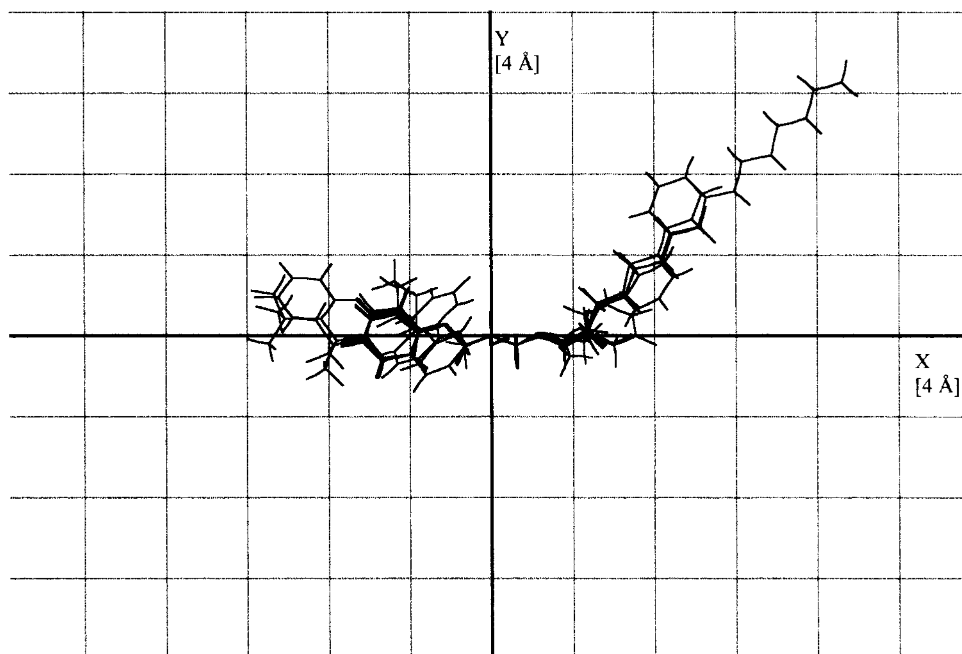


Figure 4. Superposition of substrates with $E > 50$.

residues in the 1-position such as **18**–**21**, **23**, **24**, **27**–**29**, and **33**, the enantiomer selectivity cannot be predicted and explained by Kazlauskas' rule.[14a] The reason for this behavior is hydrophobic pockets which differ not by volume but by shape. Furthermore, the presence of an acyloxy group in the 1-position has an additional stabilizing effect on the substrate fit into the active site, because the corresponding ethers **35**–**38** are acetylated with a significant lower enantiomer selectivity than the corresponding acyloxy derivatives. The amines **40** and **41** are converted by lipase PS without any enantiomer selectivity. Therefore, the beneficial effect of the acyloxy residue in the 1-position may be attributed to the carbonyl group either as an electron donator for a hydrogen bridge bond to a suitable amino acid in the binding region or to dipole–dipole interactions.

Molecular Modeling. For molecular-modeling investigations all substrates with $E > 50$ and nonsubstrates were selected. The geometry of these molecules was determined with the aid of the model build program Hyperchem Release 4.0[18] providing standard bond length and angels. Further optimization to find a local minimum was carried out with the semiempirical method AM1.[19] On the basis of the local minimum found, conformational analysis was carried out using the Conformational Search program of Chemplus Release 1.0.[18] Comparison of the low-energy conformations found by conformational analysis with the starting conformers which were found after the first geometry optimization

(18) Hypercube, Inc., Waterloo, Ontario, Canada, 1994.
(19) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909; **1993**, *115*, 5348.

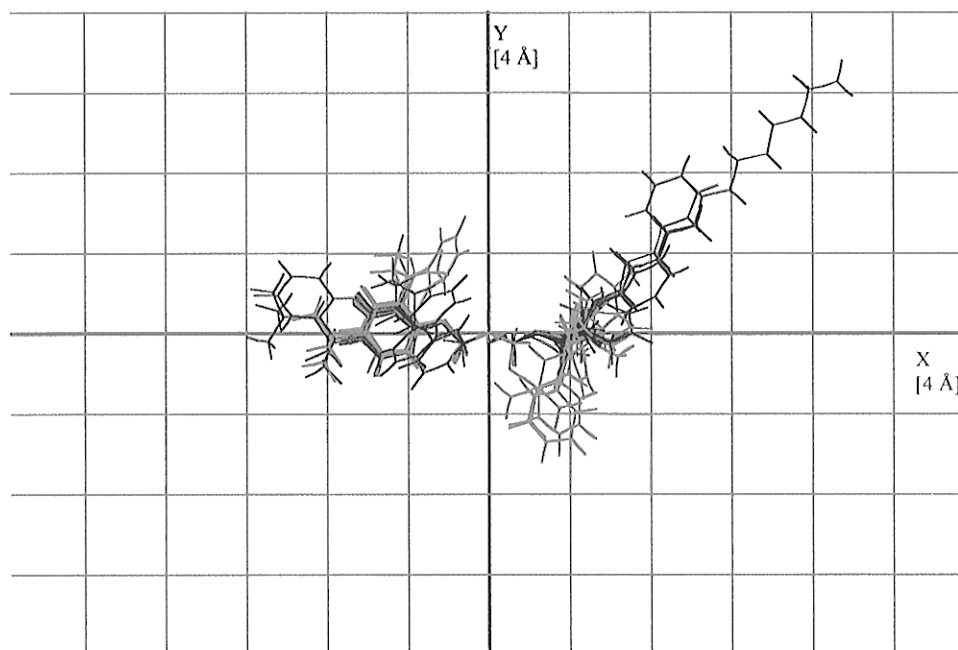


Figure 5. Superposition of substrates (black) with $E > 50$ and non-substrates (red).

with AM1 revealed that the long-stretched conformers used as starting conformers are energetically more favored than coiled conformers. Figure 3 shows 20 of the low-energy conformers of substrate **21** superimposed in the stereogenic center. A similar consideration between the conformers of all other substrates with $E > 50$ revealed that in all cases the long-stretched conformers were the most stable and by superimposing in the stereogenic center they are the only conformers with common structural regions (Figure 4). Geometry optimization for the nonsubstrates shows the same behavior. Figure 5 shows the superposition of the substrates **1**–**8**, **14**–**24**, **48**, **66**, **67**, and **69** (black) and nonsubstrates **50**–**61** (red). The stereogenic centers coincide with the origin of the coordinate system, the C–H bond with the X-axis and the C–OH bond with the *y*/*z* plane (behind the drawing plane). Figure 5 shows that the nonsubstrates do not fit to the superimposed substrates in the regions depicted in Figure 2 which were recognized as probable for a compound to be a substrate or not for the lipase from *P. cepacia*.

The correspondence of the results obtained by substrate mapping and molecular modeling clearly indicates that substrates do not fit the hydrophobic binding regions of the active site of lipase from *P. cepacia* in a coiled conformation. If this is the case, there should be no reason that the molecules identified as nonsubstrates are excluded from the lipase-catalyzed transformation. Furthermore, substrate conformations in vacuum determined by molecular modeling and of the substrates in the active site should be very similar. This is in accordance with M. J. S. Dewars' desolvation model[20] which postulates similarities between enzymatic reactions in solution and gas phase reactions.

Finally, to visualize the boundaries of the active site model of lipase PS, a modeling program[21] was used to wrap the superimposed substrates including their van der Waals radii. Figure 6 shows the patch representation of the wrapped three-dimensional active site model superimposed with the nonsubstrates and Figure 7 the simplified cubic-space representation.

(20) Dewar, M. J. S. *Enzyme* **1986**, *36*, 8.
(21) Molwrap: Overlaying of Molecules in their Stereogenic Center and Wrapping of the Superstructure, developed by Lemke, M., Berlin, Germany, 1996, access via e-mail: mtlemke@aol.com.

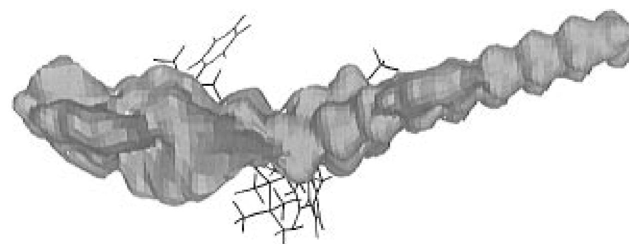


Figure 6. Superposition of wrapped substrates with unwrapped nonsubstrates.

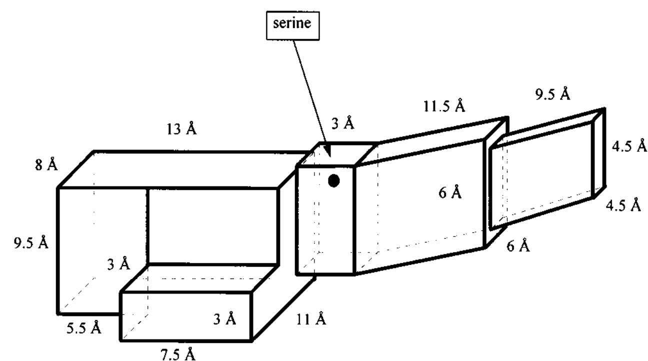


Figure 7. Simplified cubic-space model of the wrapped substrates.

Summary and Conclusion

A predictive three-dimensional active site model for lipase from *P. cepacia* was developed by substrate mapping and molecular modeling. This model clearly indicates that substrate binding and orientation is caused by two hydrophobic pockets which are very different by shape—a tube-like stretched with a very limited diameter

near the active serine and a second with a spherical shape near the active serine. High enantiomer selectivity is the result of an optimal orientation of the secondary hydroxy group of the faster reacting enantiomer in the direction of the catalytic triade of this enzyme which is caused by an optimal stabilization of the substituents adjacent at the stereogenic center. The more spherically-shaped pocket preferentially accommodates substituents such as phenyl, phenoxymethyl, *para*-substituted phenoxymethyl, (trityloxy)methyl.[7a] (*tert*-butyloxy)methyl,[4c] (phenylthio)methyl,[4c] ((*tert*-butyldimethylsilyl)oxy)methyl,[4d] or (*p*-tosyloxy)methyl[7k] which act as anchoring groups whereas the tube-like pocket hosts stretched substituents such as acetoxymethyl up to at least (*n*-hexadecanoyloxy)methyl, methyl,[4c,d,7a] ethyl,[4c,d,7a] or vinyl.[4d,7k] Otherwise, two carbon chains of quite different length do not allow an efficient stereodifferentiation between both enantiomers because a long-stretched substituent is not able to anchor the substrate in the more sherical pocket. Nonsubstrates are characterized by two sterically demanding substituents at the stereogenic center. Therefore, these compounds do not fit into the active site.

High enantiomer selectivity in lipase PS-catalyzed reactions can be predicted if secondary alcohols or their esters possess substituents at the stereogenic center which are very different by shape and not by volume.

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Supporting Information Available: Experimental and spectroscopic data for the kinetic resolution of the compounds **62**−**69** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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