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Enantioselective Acylation of Alcohols Catalyzed by Lipase QL from *Alcaligenes sp.*: A Predictive Active Site Model for Lipase QL to Identify the Faster Reacting Enantiomer of an Alcohol in this Acylation

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Abstract: Lipase QL-catalyzed acylation of secondary alcohols using isopropenyl acetate as the acylating agent in diisopropyl ether gave preferentially the corresponding acetate with an *R* configuration. On the basis of the results, a predictive active site model for lipase QL is proposed for identifying which enantiomer of a secondary alcohol reacts faster in this reaction. Copyright © 1996 Elsevier Science Ltd

Enzymes have simplified the preparation of some homochiral compounds of synthetic value.[1] In particular, lipases are attractive for this purpose because they are relatively inexpensive, can function in both organic and aqueous solutions, are simple to use and accept a broad range of substrates. There have been many reports describing the asymmetric and enantioselective synthesis of homochiral alcohols and esters by lipase-catalyzed reactions. However, there are the considerable differences in the substrate specificity between lipases and, in order to apply lipases widely to organic synthesis as chiral catalysts, it is desirable to reveal the substrate specificity for each lipase. In this regard, some active-site models or simple rules for predicting which compound is accepted as a substrate have been introduced.[2] Here we report the enantioselective acylation of secondary alcohols catalyzed by lipase QL from *Alcaligenes sp.* and, on the basis of the results, propose a predictive active site model for this lipase to identify the substrate which is accommodated in the active site and the faster-reacting enantiomer of alcohols in this acylation. An active site model for an enzyme should be a rule of thumb for judging the absolute configuration of substrates on the basis of the enantiomer selectivity of the enzymatic reaction.

Lipase QL[3]-catalyzed acylations of alcohols were carried out with isopropenyl acetate as the acylating agent in diisopropyl ether at 30 °C and the progress of the reaction was monitored by GLC. After the reaction was terminated at, or close to, the 50% conversion by removal of the enzyme by filtration, the products were separated on column and/or thin layer chromatography. The enantiomeric excess (e.e.) values of acetates and alcohols isolated were determined by HPLC using a chiral column. The absolute configurations of products were confirmed by comparison of specific rotations with those of known compounds in the literature. The results are summarized in Table 1.

From the results, we propose the active site model for lipase QL on the basis of the sizes of the groups at the stereogenic center of the substrate. For our model we use a working hypothesis that the hydroxy group being acylated is always positioned at the catalytic site and, in the case of secondary alcohols, two hydrophobic groups at the stereogenic center are located at the back of the large hydrophobic binding site (HL) because the small hydrophobic binding site (HS) cannot accommodate the methyl group. In all cases of the present reactions, the secondary alcohols with an *R* configuration were preferentially acylated and a priority of the 'large' hydrophobic group of the secondary alcohols examined here is higher than that of the

'small' hydrophobic group. On the basis of these results, we speculate that the right pocket (HL_R) of the large hydrophobic binding site is larger than the left pocket (HL_L) as shown in Figure 3.

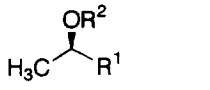
Table 1. Lipase QL-catalyzed Acylation of Secondary Alcohols

Entry	Substrate	Reaction time (h)	Conversion (%)	Products						
				Alcohol	$[\alpha]_D$	E.e. (%)	Acetate	$[\alpha]_D$	E.e. (%)	*E* value[f]
1	(±)-**1**	1.2	52	(*S*)-**1**	+4.02[b]	44	(*R*)-**2**	-1.94[c]	40	3
2	(±)-**3**	0.7	45	(*S*)-**3**	+4.49[b]	22	(*R*)-**4**	-1.57[c]	27	2
3	(±)-**5**	1.5	43	(*S*)-**5**	+2.78[b]	63	(*R*)-**6**	-6.57[c]	85	24
4	(±)-**7**	7	50	(*S*)-**7**	-36.1[b]	84	(*R*)-**8**	+105[c]	84	30
5	(±)-**9**	40	54	(*S*)-**9**	-27.7[b]	83	(*R*)-**10**	+91.8[c]	72	16
6	(±)-**11**	63	39	(*S*)-**11**	-15.8[b]	49	(*R*)-**12**	+75.5[c]	76	20
7	(±)-**13**	74	33	(*S*)-**13**	-10.8[b]	46	(*R*)-**14**	+75.2[c]	94	51
8	(±)-**15**	7 days	45	(*S*)-**15**	+11.0[b]	41	(*R*)-**16**	-17.1[c]	51	5
9	(±)-**17**	21 days	47	(*S*)-**17**	-23.0[b]	55	(*R*)-**18**	+48.5[c]	62	7
10	(±)-**19**	4	39	(*S*)-**19**	-17.1[b]	59	(*R*)-**20**	+70.7[c]	93	50
11	(±)-**21**	3	42	(*S*)-**21**	-18.6[b]	71	(*R*)-**22**	+96.8[c]	98	200
12	(±)-**23**	10	40	(*S*)-**23**	-19.4[b]	66	(*R*)-**24**	+101[c]	99	400
13	(±)-**25**	9	43	(*S*)-**25**	-26.2[b]	64	(*R*)-**26**	+11.4[c]	84	22
14	(±)-**27**	3	43	(*S*)-**27**	-19.2[b]	55	(*R*)-**28**	+68.7[c]	94	56
15	(±)-**29**	10	40	(*S*)-**29**	-23.1[b]	70	(*R*)-**30**	+92.8[c]	99	400
16	(±)-**31**	22	41	(*S*)-**31**	-21.6[b]	67	(*R*)-**32**	+92.4[c]	98	200
17	(±)-**33**	72	37	(*S*)-**33**	-22.3[b]	55	(*R*)-**34**	+116[c]	94	56
18	(±)-**35**	4.5	48	(*S*)-**35**	+13.6[b]	75	(*R*)-**36**	+84.8[c]	80	20
19	(±)-**37**	24	36	(*S*)-**37**	+14.8[b]	52	(*R*)-**38**	+105[c]	92	40
20	(±)-**39**	1	51	(1*S*,2*S*)-**39**		38	(1*R*,2*R*)-**40**	-27.6[c]	52	5
							(1*R*,2*R*)-**41**	-12.1[c]	91	
21	(±)-**42**	7 days	39	(1*R*,2*S*)-**42**	+31.9[d]	63	(1*S*,2*R*)-**43**	-29.9[d]	98	190
22	**44**	3	>98				(1*R*,2*S*)-**45**	-1.72[c]	34	
23	(±)-**46**	64	39	(*S*)-**46**	-23.0[b]	62	(*R*)-**47**	+109[c]	97	120
24	(±)-**48**	90	29	(*S*)-**48**	-26.2[b]	39	(*R*)-**49**	+48.1[c]	96	72
25	(±)-**50**	3	35	(*S*)-**50**	-25.1[c]	50	(*R*)-**51**	+116[c]	93	45
26	(±)-**52**	3	44	(*S*)-**52**	-25.7[c]	75	(*R*)-**53**	+82.6[c]	97	150
27	(±)-**54**	31	43	(2*S*,6*S*)-**54**	-46.5[e]	72	(2*R*,6*R*)-**55**	+58.8[c]	90	40
							(2*R*,6*R*)-**56**	+43.7[c]	55	

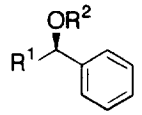
a) $[\alpha]_D$-values were measured at ambient temperature and are given in units 10^{-1} deg cm^2 g^{-1}.
b) in a methanol solution; c) in a chloroform solution; d) in a benzene solution; e) in an ethanol solution
f) *E*-Values were calculated according to the equation described in the literature.[4]

Next, in order to predict the substrate which can be accommodated in the active site of this lipase, we estimate approximately the sizes of the hydrophobic pockets. The acylations of the substrates **50** and **52** proceeded smoothly with rather high enantioselectivity but **57** was inert. The results suggest that the HL_R pocket is wide enough to accommodate the phenanthryl group but does not accept the 2,4,6-trimethylphenyl group as shown in the binding orientations (Fig 3a, 3b, and 3e). The alcohols **13** and **15** having the isopropyl group and the benzyl group as the 'small' group, respectively, were converted into the corresponding

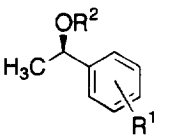
R acetates but alcohols **58** and **59** were inert. From the observations it is assumed that the HLI pocket of lipase QL is a little wider than that of lipase from *Pseudomonas fluorescens*[5] (Fig 3c, 3d, and 3f). The structure of the carbon framework of the substrates examined here is near-planar but three dimentional cage-shaped compound **54** was also acylated with moderate E-value. The stereochemistry predicted by our model is consistent with the results of the lipase PLC (*Alcaligenes sp.*)-catalyzed acylation of 2,2,2-trifluoro-1-naphthylethanols reported previously by Kato and co-workers.[6]



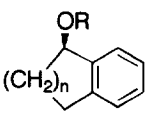
1 R^1=*n*-C_6H_{13} R^2=H
2 R^1=*n*-C_6H_{13} R^2=Ac
3 R^1=$C_3H_6NO_2$ R^2=H
4 R^1=$C_3H_6NO_2$ R^2=Ac
5 R^1=cyclohexyl R^2=H
6 R^1=cyclohexyl R^2=Ac



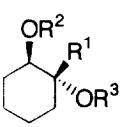
7 R^1=CH_3 R^2=H
8 R^1=CH_3 R^2=Ac
9 R^1=C_2H_5 R^2=H
10 R^1=C_2H_5 R^2=Ac
11 R^1=*n*-C_3H_7 R^2=H
12 R^1=*n*-C_3H_7 R^2=Ac
13 R^1=*i*-C_3H_7 R^2=H
14 R^1=*i*-C_3H_7 R^2=Ac
15 R^1=CH_2Ph R^2=H
16 R^1=CH_2Ph R^2=Ac



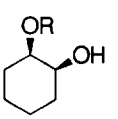
17 R^1=*o*-CF_3 R^2=H
18 R^1=*o*-CF_3 R^2=Ac
19 R^1=*m*-CF_3 R^2=H
20 R^1=*m*-CF_3 R^2=Ac
21 R^1=*m*-F R^2=H
22 R^1=*m*-F R^2=Ac
23 R^1=*p*-NO_2 R^2=H
24 R^1=*p*-NO_2 R^2=Ac
25 R^1=*p*-OCH_3 R^2=H
26 R^1=*p*-OCH_3R^2Ac
27 R^1=*p*-CF_3 R^2=H
28 R^1=*p*-CF_3 R^2=Ac
29 R^1=*p*-F R^2=H
30 R^1=*p*-F R^2=Ac
31 R^1=*p*-Br R^2=H
32 R^1=*p*-Br R^2=Ac
33 R^1=*p*-CH_3 R^2=H
34 R^1=*p*-CH_3 R^2=Ac



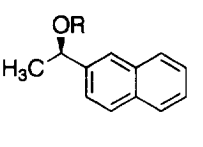
35 n=1 R=H
36 n=1 R=Ac
37 n=2 R=H
38 n=2 R=Ac



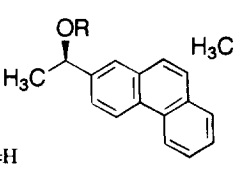
39 R^1=H R^2=R^3=H
40 R^1=H R^2=Ac R^3=H
41 R^1=H R^2=R^3=Ac
42 R^1=Ph R^2=R^3=H
43 R^1=Ph R^2=Ac R^3=H



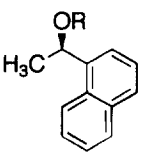
44 R=H
45 R=Ac



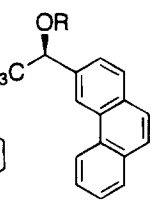
46 R=H
47 R=Ac



50 R=H
51 R=Ac



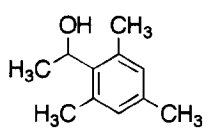
48 R=H
49 R=Ac



52 R=H
53 R=Ac

54 R^1=R^2=H
55 R^1=H R^2=Ac
56 R^1=R^2=Ac

Figure 1. The absolute configurations of faster-reacting enantiomers of alcohols are shown.



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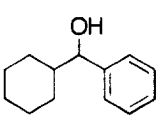

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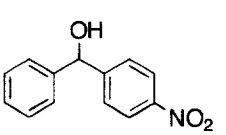
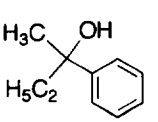


59



60

Figure 2. The alcohols **57**, **58**, **59**, and **60** were inert.

The acylations of the alcohols possessing the phenyl moiety having a polar group or an electronegative atom at its *para* or *meta*-position gave the corresponding acetate in a high enantiomeric purity. The facts demonstrate that the HLr pocket is more polar in character and the moieties having polar groups were favorably accommodated in this pocket resulting in high enantiodiscrimination.

The model described here is a rule of thumb to identify which enantiomer of a secondary alcohol reacts faster in the acylation catalyzed by lipase QL and shows that both pockets (HLr and HLl) of the large hydrophobic binding site are wider than those of lipase from *Pseudomonas fluorescens* and therefore this

lipase can be widely used for preparing homochiral alcohols.

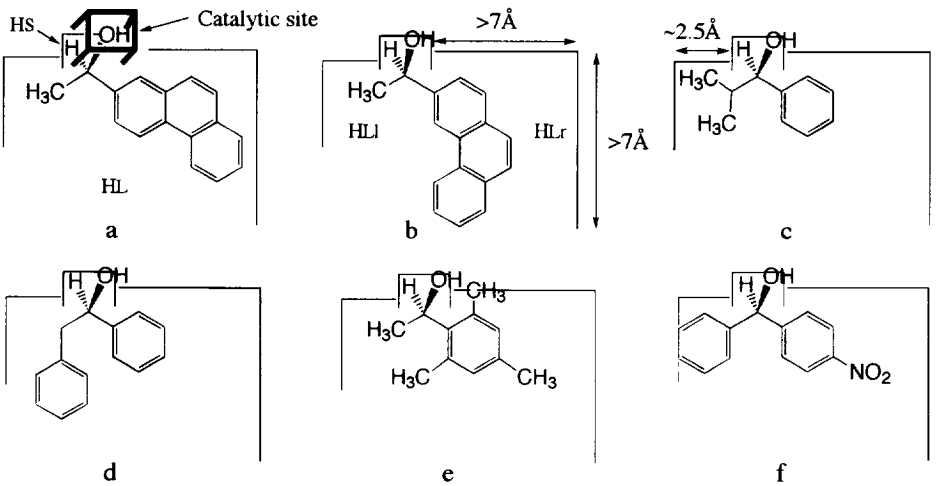


Figure3. Top perspective view of the active site model. HLl and HLr are left and right pocket of the large hydrophobic binding site, respectively. (a), (b), (c), and (d) favorable fit of secondary alcohols (*R*)-**50**, (*R*)-**52**, (*R*)-**13**, and (*R*)-**15** into the active site; (e) and (f) secondary alcohols **57** and **59** were not accommodated in the active site.

Acknowledgements

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References and Notes

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3. Lipase QL was supplied by Meito Sangyo and used without further purification.
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