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Investigation of the Impact of Water on the Enantioselectivity Displayed by CALB in the Kinetic Resolution of δ -Functionalized Alkan-2-ol Derivatives

Bin Yang, Richard Lihammar, and Jan-E. Bäckvall^{*[a]}

Abstract: It is shown that the low enantioselectivity of CALB-catalyzed transesterification of a δ -functionalized alkan-2-ol to its acetate does not correlate at all with the high enantioselectivity of the CALB-catalyzed hydrolysis of the corresponding acetate in water. This lack of correlation is unusual and for unfunctionalized alkan-2-ol derivatives there is a very good correlation between the enantioselectivity of transesterification of the alcohol and hydrolysis of the corresponding acetate ($E > 200$ in both cases). The results confirm previous predictions from molecular modeling. The water effect was mimicked by CALB variant Ala281Ser, which showed an enhanced enantioselectivity in transesterification of δ -functionalized alkan-2-ols compared to wild type CALB.

Introduction

The synthesis of enantiomerically enriched compounds is one of the most important tasks in modern organic chemistry and as a result a wide range of catalytic asymmetric synthetic methods have been developed.^[1] Among these, the kinetic resolution (KR) of racemic mixtures is a common method to prepare enantiomerically pure compounds in industry.^[2] KR is based on the different reaction rates that two enantiomers have when subjected to a chiral catalyst or reagent. Often an enzyme is employed as a chiral catalyst, where the chiral environment of the enzyme's active site leads to different binding modes of the two enantiomers during the catalysis.^[3] Lipases, which are the most commonly used enzymes in enzymatic KR, catalyze both the formation and hydrolysis of esters, often with high enantioselectivity.^[4] The enantiomer that is favored by a lipase can often be predicted by Kazlauskas' rule considering the size of the substituents at the stereogenic centre. For CALB the (*R*)-enantiomer will be preferred when the large group has a higher priority according to the CIP rule (Figure 1).^[5] Enantioselectivity is mainly quantified by the enantiomeric ratio E , which is the ratio of the rate of the *R*- and *S*-enantiomer.^[6]

The catalytic mechanism of lipases is built up by the residues of three amino acids: a nucleophilic serine, a histidine, and either an aspartate or a glutamate. In a lipase-catalyzed hydrolysis, the serine of this catalytic triad reacts with the ester substrate, forming a charged serine-bound tetrahedral

intermediate (TI). The serine-bound intermediate splits up to an alcohol and an acyl-serine, which is subsequently attacked by a water molecule releasing an acid and liberating the serine for a new round of hydrolysis. The tetrahedral intermediate is of central importance in ester hydrolysis and the rate of the reaction is enhanced due to a stabilization of this intermediate (Hydrolysis, Scheme 1). Previous research on CALB-catalyzed hydrolysis of esters has shown that the energy differences between the TI and the transition state for reaching the TI is very small. The difference in activation energy for the enantiomers can therefore be approximated to the energy difference between the corresponding two tetrahedral intermediates since the ground-state free energy of the two enantiomers is the same.^[7]

Figure 1. The preferred enantiomer in a lipase catalyzed transesterification or hydrolysis involving sec-alcohols and sec-acetate can be predicted by Kazlauskas' rule.^[5]

Interestingly, for the reverse reaction, the esterification, including transesterification, the rate limiting step is the formation of the very same tetrahedral intermediate. Also in this reaction it has been shown that the selectivity of the reaction can be rationalized as the difference in energy between the TI of the *R* and *S* substrate.^[8] Therefore, when neglecting solvent effects, the stereoselectivity of the transesterification of an alcohol to an ester should be approximately the same as that of the hydrolysis of the same ester. This hypothesis is further verified by comparing published results of lipase-catalyzed hydrolysis and transesterification reactions involving the same alcohol.^[9]

Scheme 1. Hydrolysis and transesterification proceed through the same TI

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Candida Antarctica lipase B (CALB) is a lipase that has been widely used in kinetic resolution of secondary alcohols. Its catalytic triad is built up by Ser105, His224, and Asp187 and the charged TI is stabilized by the Gln106 and Thr40. The binding site is narrow and for compounds that have a clear size difference between the large and medium groups, CALB generally shows excellent enantioselectivity with minimal to no reaction of the slow-reacting enantiomer. However, previous work in our group has shown that the selectivity of CALB-catalyzed transesterification of secondary alcohols having a keto or acetoxy group in the δ -position to the alcohol is very low.^{[10],[11],[12]} Of several investigated 5-keto-alkan-2-ols, none showed an *E* value larger than 10 in a transesterification (Scheme 2). This should be compared to the unsubstituted alkan-2-ols, which typically have large *E* values of up to 500 (*vide infra*, Scheme 3). When an acetoxy group is present in the δ -position of an alkan-2-ol the enantioselectivity decreases. Interestingly, the selectivity is highly dependent on the relative configuration between the acetoxy group and the alcohol. For *anti*-5-acetoxy-2-hexanol the *E* value is as low as 7, whereas its *syn* disasteromer has an *E* value of 94.

Scheme 2. Previously described transesterification of δ -substituted secondary alcohols

To gain a deeper understanding of the loss of selectivity in these δ -substituted alkan-2-ols we have previously made a computational study by molecular dynamic simulations of various δ -functionalized alcohols.^[13] CALB's enantioselectivity for a series of δ -substituted secondary alcohols was investigated based on the geometric properties of their serine-bound tetrahedral intermediate. It was found that for wild type CALB, δ -keto and δ -acetoxy groups on the substrate create a repulsive interaction between the δ -substituent and Thr40, shifting the position of both substrate enantiomers out of the stereogenic pocket into a non-discriminating bonding. However, molecular modeling showed that by introducing a water molecule close to Thr40, a hydrogen bond was established. This hydrogen bond brought the stereogenic center of the substrate into the stereospecific pocket, thus enhancing the stereoselectivity of the catalytic reaction. The transesterification, which is made in an anhydrous environment, cannot establish this bridging effect, eliminating the anchoring of the substrate, and as a result a low stereoselectivity is obtained.

The proposed mechanism from the molecular modelling^[13] in which water would retain the enantioselectivity for δ -keto- and δ -acetoxy-alkan-2-ols has so far neither been confirmed nor ruled out by experiments. One way to test this hypothesis would be to compare the enantioselectivity of the CALB-catalyzed transesterification of the δ -keto- and δ -acetoxy-alkan-2-ols to their acetates with the CALB-catalyzed hydrolysis of the

corresponding 2-acetoxy derivatives of the δ -keto- and δ -acetoxy-alkan-2-ols. The transesterification would be carried out in a dry organic solvent whereas the hydrolysis would be carried out in water. In this communication we report that the hydrolysis of the esters of the δ -keto- and δ -acetoxy-alkan-2-ols occurs with high enantioselectivity.

Result and Discussion

To verify that the correlation in selectivity between the transesterification and the hydrolysis does exist for compounds lacking a δ -substituent, we performed the enantioselective transesterification and hydrolysis of compound *rac*-2a and *rac*-1a in toluene and aqueous buffer solution, respectively (Scheme 3). In both cases CALB was active and displayed very high enantioselectivities (*E* > 200).

Scheme 3. CALB catalyzed transesterification and hydrolysis of compound *rac*-1a and *rac*-2a

In order to study the effect of the δ -functional group, compounds *rac*-1b–1f and *rac*-2b–2f were synthesized (Figure 2). The preparation of diastereomerically pure racemic 1,4-*anti* compounds *rac*-1b–1c and *rac*-2b and *rac*-2c is far from trivial since the *syn* and *anti* diastereomers most often cannot be separated by simple chromatography. Instead selective synthesis of the corresponding racemic *anti*-diol is required in order to obtain these compounds diastereomerically pure. The diol can later be transformed into the mono- and di-acetates by simple acetylation.

Figure 2. Substrate scope for hydrolysis and transesterification reactions

The synthesis of the *anti*-1,4 diol precursors required for *rac*-1b and *rac*-2b was achieved by the method described by Knapp et al. utilizing a diastereoselective BH_3 -mediated rearrangement of 1-substituted 2-methyl-cyclobutenes (Scheme 4).^[14]

The ring-opening of **3** with $\text{BH}_3 \cdot \text{THF}$ (1.1 equiv) gave an intermediate borolane, which on treatment with $\text{NaOH}/\text{H}_2\text{O}_2$ afforded racemic *anti*-1,4-diol **4** as the major product in 82% yield with high diastereoselectivity. Compound *rac*-4 was then converted to *rac*-1b and *rac*-2b using 2 or 1 equiv., respectively, of acetic anhydride in an acetylation reaction.

Scheme 4. Synthesis of *rac*-1b and *rac*-2b

Racemic *anti*-diol **8**, required for the synthesis of acetates *rac*-1c and *rac*-2c, was acquired by a strategy described by Fujioka (Scheme 5).^[15] The synthesis starts with haloetherification reaction of acetal *rac*-5 in the presence of *N*-iodosuccinimide (NIS) and 2-methoxyethanol, followed by a LiAlH₄ reduction giving a mixture of cyclic acetals from which *rac*-6 could be separated from its diastereomers by silica gel column chromatography in 52% yield over two steps. In the next step, the substitution of the 2-methoxyethoxy group by an *n*-butyl group was achieved with full retention of stereochemistry yielding *rac*-7 in 78% yield.^[16] Hydrogenation of *rac*-7 afforded the *rac*-anti-diols **8** in 99% yield, which was converted to *rac*-1c and *rac*-2c using 2 or 1 equiv., respectively, of acetic anhydride in an acetylation reaction.

δ -Keto alcohols *rac*-2d to *rac*-2f were obtained according to known procedures^[17] and the corresponding δ -ketofunctionalized acetates *rac*-1b to *rac*-1f were acquired by chemical esterification using acetic anhydride.

The stereoselectivity of CALB-catalyzed transesterification of δ -substituted alcohols *rac*-2b and *rac*-2f was studied at 30 °C employing 2 equiv. of isopropenyl acetate as acylating agent and toluene as solvent. For *anti*-acetoxyl substrates *rac*-2b and

Scheme 5 Synthesis of *rac*-1c and *rac*-2c

rac-2c the reaction showed low enantioselectivity with *E* values of 18 and 36, respectively (Table 1, Entry 1-2), whereas for δ -keto substituted alcohols *rac*-2d, *rac*-2e and *rac*-2f CALB displayed poor selectivity with *E* values under 10 (Entry 3-5). Next, we set out to investigate the reverse reaction, i.e. the hydrolysis of the corresponding acetates. The hydrolysis was performed at 30 °C in a phosphate buffer solution (50 mM, pH 7.0) with a liquid preparation of CALB. Diacetates *rac*-1b and *rac*-1c were, as expected, only hydrolyzed at the C4 carbon (to give *rac*-2b and *rac*-2c, respectively) and the reaction showed enantio-selectivities which were superior to those of the transesterification reaction (Table 1). The same trend was observed for δ -keto-alcohols *rac*-1d to *rac*-1f where the *E* values in all cases were ≥ 170 . When comparing the transesterification and hydrolysis of the δ -functionalized alkan-2-ol derivatives, the *E* values of the hydrolysis are 13-100 times larger than the *E* values for the corresponding transesterification. The extreme difference in enantioselectivity that CALB displays for these compounds is remarkable since the *E* value observed in transesterification and hydrolysis normally are of the same

Table 1 CALB-catalyzed transesterification of δ -functionalized alcohol and hydrolysis of the corresponding δ -functionalized esters

Entry	Substrate	Transesterification ^a				Hydrolysis ^b				
		conv. (%)	ee _{OH} (%)	ee _{OAc} (%) ^c	<i>E</i> value	Substrate	conv. (%)	ee _{OAc} (%) ^c	ee _{OH} (%)	<i>E</i> value
1	<i>rac</i> -2b	36 ^d		84.1	18 ^e	<i>rac</i> -1b	30 ^f	42.0	99.8 ^g	>500 (1512) ^h
2	<i>rac</i> -2c	33 ^f	44.4 ^c	91.6	36 ^h	<i>rac</i> -1c	40 ^f	66.2	99.2 ^c	331 ^h
3	<i>rac</i> -2d	23 ^d (29 ^{d,i})		76.1 (93.7) ⁱ	9 ^e (45 ^{e,i})	<i>rac</i> -1d	42 ^f	71.6	97.5 ^g	170 ^h
4	<i>rac</i> -2e	34 ^f	23.6 ^g	45.7	3 ^h	<i>rac</i> -1e	47 ^f	89.3	98.6 ^g	431 ^h
5	<i>rac</i> -2f	47 ^d		55.5	6 ^e	<i>rac</i> -1f	48 ^d	90.0	96.8 ^g	190 ^h

a) Reaction conditions: *sec*-alcohol *rac*-2b to *rac*-2f (0.1 mmol), supported CALB, (Novozyme 435, 40 mg/mmol), isopropenyl acetate (2 equiv.), Na₂CO₃ (10 mg) in toluene (0.2 ml), performed at 30 °C. b) Reaction conditions: acetate *rac*-1b to *rac*-1f (3 mg), CALB solution (Novozymes CALB, aqueous solution, 4 μ L, Minimum 5,000 LU/G of liquid), in KPO₄ buffer (50 mM, pH 7.0)/MeCN (85:15), shaken with the speed of 290 rpm at 30 °C. c) Determined by GC. d) Determined by ¹H NMR. e) Calculated from conversion and ee_{prod} according to Sih's formula^[18]. f) Conversion was calculated based on ee_{OAc} and ee_{OH}^[18]. g) In order to determine ee_{OH} by GC, the alcohol was isolated and esterified by Ac₂O and DMAP. h) Calculated from the ee_{prod} and ee_{subs} according to Sih's formula.^[18] i) reaction performed as previously, but the solvent toluene is saturated with water (*a_w* = 1)

magnitude (cf. Scheme 3 and Table S1). Clearly, in an anhydrous environment, the δ -functionality forces the substrate to adopt a binding mode tilted away from the stereogenic pocket, making the influence from the active site less pronounced. However, in an aqueous environment this effect is cancelled and the enantioselectivity is restored. These findings support our previous hypothesis that a water molecule around Thr40 can anchor the substrate in the stereospecific pocket and thereby reduce the influence of the repulsion between the enzyme backbone and the δ -substituent. Interestingly, when the transesterification of substrate **2d** was performed in toluene saturated with water ($a_w = 1$) an increase of the E value from 9 to 45 (entry 3, Table 1) was observed. This increase in enantioselectivity further supports our hypothesis concerning the effect by water.

In our previous molecular modelling calculations it was predicted that CALB variant Ala281Ser, where alanine in position 281 is changed to a serine, would create a mimic of the water molecule that may appear in the active site around Thr40. The latter mutant is therefore expected to restore the enantioselectivity in the transesterification of the δ -functionalized alkan-2-ols.^[13] We have now experimentally tested CALB A281S, prepared via site directed mutagenesis, on some of the substrates used in the present study. The enantioselectivity of transesterification towards δ -functionalized ketoalcohols **rac-2d-2f** was measured with CALB A281S supported on Celite. The enantioselectivity for transesterification of δ -keto-alkan-2-ols had

It was also demonstrated that variant Ala281Ser showed a high E value ($E = 118$) in the hydrolysis of (*rac*)-**1d** (Eq 1). Thus, this mutant has a high enantioselectivity in both directions (hydrolysis and transesterification).

In conclusion, CALB-catalyzed hydrolysis and transesterification reactions involving the same alcohol may display vastly different enantioselectivities (E values) for certain δ -functionalized alkan-2-ol derivatives. The results suggest that hydrolysis of δ -functionalized alkan-2-ol esters in water do not proceed through the same enantiodetermining tetrahedral intermediate as the transesterification of the δ -functionalized alkan-2-ol in an organic solvent. Our results are in accordance with the assumption that a water molecule around Thr40 allows the enzyme to restore its enantioselectivity for the δ -functionalized alkan-2-ols involved. The effect of a water molecule can be partly mimicked by replacing alanine at position 281 with serine and it was found that the selectivity of CALB A281S is enhanced for δ -keto-alkan-2-ol compared to that of wt CALB.

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Keywords: Lipase • CALB • Enantioselectivity • Hydrolysis • Transesterification

Table 2 Transesterification catalyzed by CALB A281S and wild type CALB^a

Entry	Substrate	Product	E value ^b A281S	E value ^b wt CALB	Selectivity increase (%)
1	<i>rac</i> - 2d	(<i>R</i>)- 1d	115	9	1278
2	<i>rac</i> - 2e	(<i>R</i>)- 1e	19	3	633
3	<i>rac</i> - 2f	(<i>R</i>)- 1f	19	6 (3 ^c)	317

a) Reaction conditions: (i) for CALB A281S; sec-alcohol *rac*-**2d** to *rac*-**2f** (0.1 mmol), supported CALB A281S (on Celite, 40 mg/mmol), isopropenyl acetate (2 equiv.), Na₂CO₃ (10 mg) in toluene (0.2 ml), performed at 30 °C, reaction time 24 h. (ii) for wt CALB; sec-alcohol *rac*-**2d** to *rac*-**2f** (0.2 mmol), supported CALB (Novozyme 435, 40 mg/mmol), isopropenyl acetate (2 equiv.), Na₂CO₃ (10 mg) in toluene (0.2 ml), performed at 30 °C. A control experiment with wild type CALB supported on Celite was performed on **2f**, which gave an E value of 3. b) Calculated according to the formula by Sih et al.^[18] c) CALB on celite.

improved significantly (Table 2). However, for the anti-acetoxyalcohols *rac*-**2b** and *rac*-**2c** the activity of mutant A281S was too low to enable the determination of the E value. In order to verify that the enantioselectivity enhancement arises solely from the mutation and not from the immobilization onto Celite was conducted. The E value for the transesterification of *rac*-**2f** with CALB on Celite was as low as 3, confirming that wt CALB is not selective in transesterifications of these substrates.

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Entry for the Table of Contents

COMMUNICATION

CALB-catalyzed transesterification of alcohol **A** to its acetate does not correlate with the high enantioselectivity of the CALB-catalyzed hydrolysis of the corresponding acetate **B** in water. This lack of correlation is unusual and for unfunctionalized alkan-2-ol derivatives there is a very good correlation between the enantioselectivity of transesterification of the alcohol and hydrolysis of the corresponding acetate.

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