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Probing the enantioselectivity of *Bacillus subtilis* esterase BS2 for *tert*. alcohols

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ABSTRACT

The activity and enantioselectivity of several mutants of the esterase BS2 from *Bacillus subtilis* have been investigated. In the enzymatic hydrolysis of α,α-disubstituted cyanohydrin acetates, a class of *tert*. alcohol esters, they were active but not selective. In contrast to this result similar *tert*. acetylenic alcohol esters were hydrolysed with high *E*-values (>100). The difference in reactivity has been studied by molecular dynamics studies. The computer model suggested that the source of the observed difference in reactivity between the two very similar *tert*. alcohol esters lies in the ability of the cyanohydrins to form hydrogen bonds to water molecules—even when the substrate is in the active site.

1. Introduction

Recently, *Bacillus subtilis* esterase BS2 and several mutants were shown to be very active and enantioselective catalysts for the hydrolysis of *tert*. acetylenic alcohols (**2e**). Enantioselectivities (*E*) exceeding 100 were observed for mutants BS2 G105A, E188D and E188W/M193C, making these enzymes very promising candidates for the kinetic resolution of other *tert*. alcohols [1–3]. The enantioselective catalytic synthesis of *tert*. alcohols is a major challenge [4–6], thus a new tool for their preparation is highly desired. To further probe the factors determining the enantioselectivity of *B. subtilis* esterase BS2 other substrates were investigated, both with molecular modelling and by catalytic experiments. Molecular modelling had already proven to be a versatile tool to gain further insight into the molecular basis of the enantioselectivity of BS2 esterase in the hydrolysis of some substrates [3].

Structurally, the *tert*. acetylenic alcohol acetates (**2**) are closely related to α,α-disubstituted cyanohydrin acetates (**1**) (Fig. 1). Essentially only the CH of the acetylenic moiety is replaced by a nitrogen atom. The *tert*. acetylenic alcohol acetates (**2**) commonly contain CF_3 groups rather than CH_3 groups, to suppress autohydrolysis [1]. Indeed, for **2b** no enantioselective enzyme-catalysed hydrolysis is possible, due to this competing autohydrolysis. In the case of the cyanohydrin acetates (**1**) this is not necessary as they are less susceptible to autohydrolysis. Given the great importance of cyanohydrins as versatile building blocks and the limited accessibility of enantiopure ketone-based cyanohydrins [7,8], *B. subtilis* esterase BS2 and its mutants were screened for activity and enantioselectivity in the hydrolysis of α,α-disubstituted cyanohydrin acetates (**1**, Fig. 1). To date only very few hydrolases that can catalyse this transformation are known, notably the *S*-selective Subtilisin A and the *R*-selective *Candida rugosa* lipase (CRL) for aromatic substrates [9] and whole cells from *Pichia miso* and *Bacillus coagulans* strains [10,11] as well as CRL for a very limited number of aliphatic substrates [12]. Based on the structural similarities between **1** and **2**, insight into the factors determining the activity and enantioselectivity of *B. subtilis* esterase BS2 is expected from these structure activity studies.

2. Experimental

2.1. Molecular modelling

The docking experiments were performed by using AutoDock4 [13] and AutoGrid4 in combination with AutoDock-Tools [14]. In the first step, a grid box was defined ($45 \times 45 \times 45$ points with a grid

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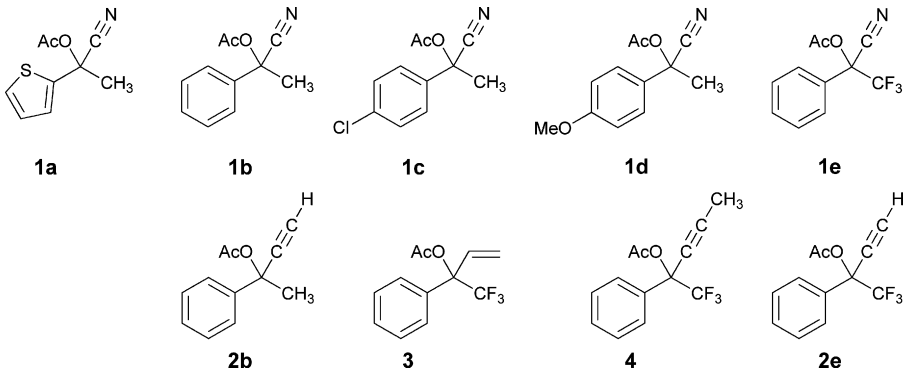


Fig. 1. *Tert.* acetylenic alcohol acetates are structurally closely related to α,α-disubstituted cyanohydrin acetates.

spacing of 0.375 Å), defining the space for the docking experiment. The box was centred on the catalytically active Ser189. The substrate enantiomers were docked into a refined structure of BS2 esterase based on the crystal structure of the highly homologous BsubpNBE (pdb-entry 1qe3 [15]). In the second step, the Larmackian–Genetic algorithm was used with max. 15 million energy evaluations and max. 2700 generations. 100 independent runs were performed starting with a population of 150 individuals. Three of the five possible torsions of the substrate were active with 20°/step. All other options were set on program defaults. The non-covalently docked binding modes were screened for productive orientations in which the distances and angles from the atoms of the functional group of the substrate to the catalytic residues (e.g. Ser189, His399 and the oxyanion hole Gly106, Ala107 and Ala190) would allow for the formation of the tetrahedral intermediate. These binding modes were used as a basis for the respective tetrahedral intermediates according to a previous study [3].

Molecular dynamic studies were performed in a periodic water box using the YASARA [16] software (version 7.4.22) with AMBER99-forcefield using long-range electrostatics with a cut-off at 7.86 Å (Particle-Mesh-Ewald) [17]. The force field of the substrate was obtained using AutoSMILES force field parameter assignment [18]. After addition of the solvent, cell neutralization and pKs-prediction [16] simulations of 500 ps were performed at 35 °C, pH 7.5 and a solvent density of 0.997 g/L. Each simulation step contained a 1.25 fs step for inter- and intramolecular forces. Frames were saved each 3000 time steps. Descriptors for hydrogen bonds between water molecules and the nitrile group were set as: maximal distance HOH–NC $d < 2.6$ Å; minimal angle HOH–NC $\theta > 120°$; minimal angle HOH–NC $\theta > 120°$. The optimized starting structures of the MD simulations were created based on the results of the docking study.

2.2. Materials and methods

Ethyl acetate (>99%), 1,3,5-triisopropylbenzene (TIB, 97%, Fluka), *n*-dodecane (DOD, 99+%, Aldrich), dimethyl sulfoxide (99.7%, Acros), esterases: wild type (69 U mg^{-1}), G105A mutant (40 U mg^{-1}), E188W/M193C mutant (39 U mg^{-1}), E188D mutant (38 U mg^{-1}) and E188F mutant (55 U mg^{-1}) (from *B. subtilis* esterase BS2; lyophilised enzyme was prepared as described earlier [1–3]), potassium dihydrogen phosphate (>99%, Baker), dipotassium monohydrogen phosphate (>99%, Baker), sodium sulfate (99.0%, Merck). The enzyme activity was determined using a Shimadzu UV-2401PC spectrophotometer. The pH of the buffer was determined using a Metrohm 691 pH-meter, which was first calibrated using two stock solutions with a known pH (pH 4.0 and pH 7.0). Gas chromatography was performed using an enantioselective β-cyclodextrin column (Chirasil-Dex CB 25 m × 0.2 mm) on a Shimadzu Gas Chromatograph GC-17A equipped with a FID detector and a Shimadzu Auto-injector AOC-20i, using He as the carrier gas. The temperature programs and retention times were: **1a**: 130 °C (10 min): 4.73 min (*S*) and 5.09 min (*R*); **1b**: 110 °C (20 min): 11.90 min (*S*) and 13.86 min (*R*); **1c**: 160 °C (10 min): 3.73 min (*S*) and 3.96 min (*R*); **1d**: 150 °C (10 min): 7.57 min (*S*) and 7.94 min (*R*); **1e**: 110 °C (10 min): 3.37 min (*R*) and 3.99 min (*S*).

The absolute configuration of **1b** was determined by comparison with optical rotation and elution order of the enantiomers by enantioselective GC, as described earlier [9]. For substrates **1a**, **1c**, **1d** and **1e** analogous chromatographic behaviour was assumed.

Racemic cyanohydrin acetates **1a**–**e** were prepared by cyanation, deprotection and acetylation as described earlier. The characterization of **1a**–**d** was in accordance with the literature [9]. Spectroscopic data for trifluoroacetophenone cyanohydrin acetate (**1e**):

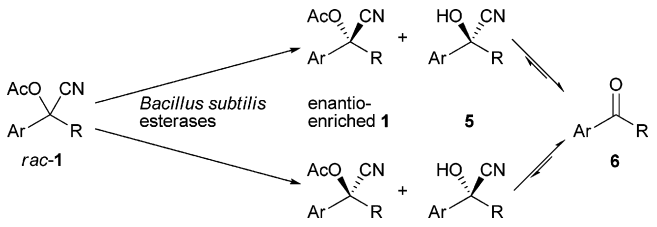
^{1}H NMR ($CDCl_3$, 400 MHz): 2.26 (s, 3H), 7.50 (m, 5H);
^{13}C NMR ($CDCl_3$, 400 MHz): 20.6 ($COCH_3$), 75.1 (q, quaternary C), 112.1 (Ar), 122.2 (CF_3), 126.3 (Ar, 2C), 128.7 (Ar), 129.2 (Ar, 2C), 135.6 (C≡N), 166.5 (C=O);
MS: 243 (M^+), 235, 217, 201, 181, 157, 134, 114, 105, 88, 77, 69, 43.

2.3. General procedure for preparing the calibration curve

For each substrate and internal standard (IS) a stock solution was prepared (100 mM in ethyl acetate). Each sample consisted of IS-solution (0.1 mL) and known amounts of substrate solution (5, 10, 12.5, 15, 17.5, 20 and 25 mM) to attain the right concentration. Ethyl acetate was added to a total volume of 1 mL. For substrate **1a**, **1b** and **1e**, TIB was used as internal standard, for substrate **1c** and **1d** DOD. The calibration curve was plotted using the peak areas of the GC analysis and the known concentrations of IS and substrate.

2.4. General procedure for enzyme activity assay

Esterase activity was determined spectrophotometrically by hydrolysis of *p*-nitrophenyl acetate (*p*NPA, 1.0 mL, 10 mM in DMSO) in phosphate buffer (2.9 mL, 100 mM, pH 7.5). Enzyme solution (0.1 mL) was used (1 mg enzyme in 2 mL buffer, for the E188W/M193C mutant: 2.3 mg in 2 mL). The blank cuvette consisted of the substrate (1.0 mL) and buffer (3.0 mL) to correct for auto-hydrolysis. *p*-Nitrophenol released was quantified ($\lambda = 410$ nm, $\varepsilon = 15 \times 10^3$ M^{-1} cm^{-1}). The path length of the cuvettes used was 1.0 cm. The assays were performed over a period of 10 min. One unit (U) of activity was defined as the amount of enzyme (mg) releasing 1 μmol *p*-nitrophenol per min.



Scheme 1. Enzymatic hydrolysis of cyanohydrin esters with esterase BS2 and its mutants.

2.5. General procedure esterase-catalysed small-scale resolution

The substrates dissolved poorly in the buffer and were thus not suitable for preparing stock solutions. To know the exact amount of substrate, it was weighted in the reaction vial for each reaction in order to achieve a solution of 25 mM in 1 mL. Phosphate buffer (0.8 mL, 100 mM, pH 7.5) was added. DMSO (0.1 or 0.2 mL as co-solvent) and enzyme solution (0.1 mL = 12 U) were added. The reaction mixture was stirred at 37 °C in a DESYRE-Mix thermo shaker (Zinsser Analytics) for a certain time (1, 2 and 4 or 20 h). Reaction times were chosen (except for the 20 h) to be able to make a direct comparison with earlier work [2,3]. Because of the low activity towards **1e**, the reaction time of this substrate was longer. The internal standard dissolved poorly in the buffer. A stock solution of internal standard (100 mM in ethyl acetate, 0.1 mL) was added to the reaction mixture after the reaction was finished to prevent the ethyl acetate from influencing the reaction. TIB was the internal standard for **1a**, **1b** and **1e**, DOD for **1c** and **1d.** The total reaction mixture was extracted twice with ethyl acetate (1.5 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under nitrogen to a volume of 1 mL. The sample was centrifuged until all solid particles were precipitated and the organic layer was transferred to a GC-vial. The method used for the work-up process was optimized to an accuracy of >90%. ee_s and conversion were determined by chiral GC analysis. From the results, the *E*-value was calculated. The product released by the enzyme decomposes immediately to the corresponding ketone and HCN. Thus, ee_p could not be determined. Substrates **1a**–**d** were already tested intensively and it had been demonstrated that they were stable in buffer over long periods of time [9]. The stability of **1e** was also checked by preparing a blank in buffer and DMSO. No reaction occurred.

3. Results and discussion

3.1. Catalytic experiments

B. subtilis esterase BS2 and its mutants G105A, E188W/M193C, E188D, E188F were employed in the hydrolysis of five α,α-disubstituted cyanohydrin acetates (**1a**–**e**, Fig. 1). To allow direct comparison the experiments were carried out as optimized for **2e**. Hydrolysis of 25 mM **1a**–**e** was performed in a 100 mM phosphate buffer (pH 7.5) containing 12 units of the indicated esterase per mL and 10% DMSO to ease substrate solubility. Under the reaction conditions free cyanohydrins **5a**–**e** will be formed. However, these compounds are not stable and immediately decompose to the corresponding ketones **6a**–**e**. Therefore only the remaining enantioenriched **1a**–**e** have been isolated and analysed (Scheme 1 and Table 1).

All enzyme variants were active and display good activity towards **1a**–**d** (Table 1). The wild-type BS2 was generally the most active enzyme, the exception being **1c** where G105A and E188W/M193C showed higher activity. For **1e** all enzymes were active, too, but conversions were slow and extended reaction times had to be used. In the absence of enzyme all α,α-disubstituted cyanohydrin acetates (**1a**–**e**) were stable and no background hydrolysis was observed.

Table 1
Enzymatic hydrolysis of cyanohydrin acetates[a].

Substrate	Enzyme	Time [h]	Conv. [%]	ee_s[b] [%*ee*] (*R*/*S*)	*E*
1a	Wild type	2	73	39 (*S*)	1.9
1a	G105A	2	63	44 (*S*)	2.5
1a	E188W/M193C	2	37	1 (*R*)	1.0
1a	E188D	2	24	4 (*R*)	1.4
1a	E188F	2	24	0 (–)	1.0
1b	Wild type	4	69	51 (*S*)	2.5
1b	G105A	2	41	36 (*S*)	4.4
1b	E188W/M193C	2	21	0 (–)	1.0
1b	E188D	4	40	36 (*S*)	4.6
1b	E188F	4	0	0 (–)	n.d.
1c	Wild type	2	28	8 (*S*)	1.7
1c	G105A	4	46	31 (*S*)	2.8
1c	E188W/M193C	2	48	0 (–)	1.0
1d	Wild type	2	55	2 (*R*)	1.1
1d	G105A	2	51	28 (*S*)	2.2
1d	E188W/M193C	2	17	0 (–)	1.0
1e	Wild type	20	26	3 (*R*)	1.2
1e	G105A	20	26	7 (*R*)	1.6
1e	E188W/M193C	20	25	2 (*R*)	1.2
1e	E188D	20	63	1 (*R*)	1.0
1e	E188F	20	62	0 (–)	1.0

[a] *Conditions*: 12 U enzyme, 25 mM substrate in 1 mL phosphate buffer (100 mM, pH 7.5), 10% (v/v) DMSO, 37 °C.
[b] *ee* of the remaining substrate.

To our surprise none of the enzyme variants displayed significant enantioselectivity. This is all the more remarkable, since all of them were active in the hydrolysis of these sterically very demanding substrates. Indeed, the enantioselectivity was always below 5, while the same enzyme variants had displayed $E > 100$ for the closely related *tert*. acetylenic alcohol ester (**2e**) [1,2].

A factor that was earlier noted to influence the activity and enantioselectivity of BS2 towards arylaliphatic *tert*. alcohol esters (**2e**) is the amount of DMSO in the reaction mixture [1]. Addition of DMSO was useful to reduce the deleterious hydrolysis of these compounds, thus increasing the enantioselectivity of the overall reaction. With an increased amount of DMSO of 20% (v/v), significantly higher activity of all esterases was observed for **1e**. However, no increase of the enantioselectivity was achieved (Table 2). For all other substrates activity and enantioselectivity were not much influenced by the increase of the DMSO concentration. The improved activity of all esterases towards **1e** may be ascribed to the improved solubility of the substrate. Substrates **1a**–**d** were already reasonable well dissolved with 10% (v/v) DMSO, explaining why no change in activity was observed.

Table 2
Influence of DMSO on activity and selectivity in enzymatic hydrolysis[a].

Substrate (% DMSO)	Enzyme	Time [h]	Conv. [%]	ee_s[b] [%*ee*] (*R*/*S*)	*E*
1a (10)	Wild type	1	51	11 (*S*)	1.4
1a (20)	Wild type	1	50	13 (*S*)	1.4
1b (10)	Wild type	1	39	15 (*S*)	1.9
1b (20)	Wild type	1	40	3 (*S*)	1.1
1e (10)	Wild type	20	26	3 (*R*)	1.2
1e (20)	Wild type	20	63	2 (*R*)	1.1
1e (10)	G105A	20	26	7 (*R*)	1.6
1e (20)	G105A	20	61	4 (*R*)	1.1
1e (10)	E188W/M193C	20	25	2 (*R*)	1.2
1e (20)	E188W/M193C	20	75	1 (*R*)	1.0

[a] *Conditions*: 12 U enzyme, 25 mM substrate in 1 mL phosphate buffer (100 mM, pH 7.5), 10% or 20% (v/v) DMSO, 37 °C.
[b] *ee* of the remaining substrate.

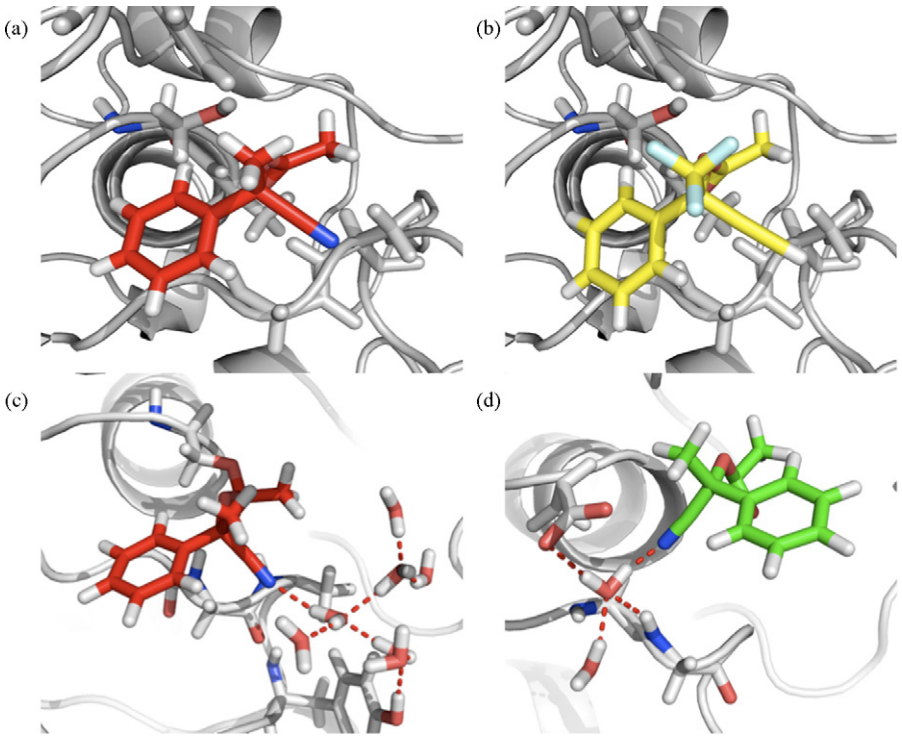


Fig. 2. (a and b) After docking, the productive binding modes of the faster reacting enantiomers (*S*)-**1b** (a) and (*R*)-**2** (b) were identical. (c and d) Snapshots of the tetrahedral intermediates (TIs) of (S)-**1b** (c) and (R)-**1b** (d) in the active site of BS2 after molecular dynamics simulations. Hydrogen bonds are highlighted as red dashes. The nitrile group of (*R*)-**1b** is less accessible to hydrogen bonding.

In previous studies dealing with similar compounds it already became clear that the acetylenic substituent plays a crucial role regarding both enantioselectivity and activity of esterases and lipases. Already in the early 90′s it was shown by O'Hagan et al. that the lipase from *C. rugosa* (also bearing the GGG(A)X motif) displays enantioselectivity towards substrates of the type **2e**, whereas an analog substrate bearing a vinyl-group (**3**), a propynyl-group (**4**) or a nitrile group (**1e**) were not converted [19,20]. These findings were confirmed later on with the esterase BS2 from *B. subtilis*. The wild-type enzyme and the two variants E188D and G105A showed moderate to very high enantioselectivity towards **2e**. The analog substrates bearing a vinyl- (**3**) or propynyl-group (**4**) were not converted.

A similar result obtained with the lipase A from *Candida antarctica* supports these observations: the lipase was active and enantioselective ($E = 65$) in the transesterification of 2-phenylbut-3-in-2-ol to give **2b**. In this case autohydrolysis could not occur and the enantioselectivity of the enzyme could be observed [21]. The analogue nitrile **1b**, however, was not converted [9].

Both, the reported data from the literature and the results using BS2 esterase indicate that lipases and esterases catalyse the conversion of these very similar substrates to a very different degree. In the case of BS2, this is very surprising as the active site is spacious and the substrates differ hardly in respect to sterical hindrance.

3.2. Molecular modelling studies

To gain further insight to the strong differences in reactivity of substrates **1a–e** compared to substrates of the type **2e**, a molecular modelling study was performed. Both enantiomers of **1b** were docked into the active site of BS2 esterase in order to identify productive binding modes. The results of this docking study have to be interpreted carefully because the docking was performed in the absence of an explicit solvent box; this represents a severe limitation as the active site of BS2 is open to the water. Nevertheless, the docking allowed to identify several possible binding modes for each enantiomer. While the majority was clearly non-productive, for each substrate enantiomer one potentially productive binding mode for each enantiomer of **1b** in the active site of BS2 esterase was found. The results were compared to a previous study dealing with arylaliphatic *tert*. alcohol acetates [3]. Interestingly, the faster reacting (*S*)-**1b** was accommodated in the same orientation as the faster reacting enantiomer of **2e**. The binding modes of the slower reacting enantiomers had different configurations. From all productive binding modes the respective tetrahedral intermediates were generated and subjected to molecular dynamics simulations of 600 ps. The results of these MD simulations of each of the two enantiomers of **1b** suggested an explanation for the observed difference in reactivity toward **2e**; a different ability of the enzyme-bound substrate to interact with the solvent.

During 600 ps molecular dynamics simulations of the tetrahedral intermediates of both enantiomers, water molecules entered the active site. Between these water molecules and the nitrile group of both enantiomers, hydrogen bonding was observed (Fig. 2). For instance, in the frame shown, the distance between the hydrogen atom of the water molecule and the acceptor nitrogen atom of (*S*)-**1b** was 2.1 Å. The angle HO*H*–*NC* was 143.8° and the angle HO*H*–*NC* 150.4°. In the case of (*R*)-**1b**, the distance was 2.1 Å, with an angle for HO*H*–*NC* of 162.9° while the angle for HO*H*–*NC* was 154.2°. The angle HO*H*–*NC* is of interest because the sp nitrogen lone electron pair is the hydrogen-bond acceptor site. Consequently, the geometry of the *HOH*–*NC* complex is quasi-linear. In MD simulations of both enantiomers, the values of 70% of the frames over the whole trajectory were in good agreement with the descriptors reported by Le Questel et al. for intermolecular hydrogen bonds of nitriles obtained both from crystallographic data and from *ab initio* calculations [22], suggesting that the enzyme-bound substrate forms hydrogen bonds to water molecules.

It was thus shown that the CN group of the enzyme-bound substrate is accessible to water molecules and that the enzyme-bound

substrates can undergo hydrogen bonding in the case of both enantiomers. In addition to the enthalpic effect of a hydrogen bond, the connection to the solvent box also may have a strong effect on the flexibility of the substrate in the active site and thus on the entropy contribution. In contrast, the ethynyl group of the analogue **2e** cannot serve as hydrogen-bond acceptor. A role of the ethynyl group as hydrogen donor in a hydrogen bond CCH–OH_2 would be a tempting thought. However, with a pK_a of 25, the acetylene group can be regarded as a rather weak hydrogen bond donor.

The effect of the solvent composition on enzyme enantioselectivity has mainly been attributed to conformational changes on the enzyme structure. Ke and Klibanov could rule out these effects by using enzyme crystals as catalysts. They explained a strong dependence of the enantioselectivity of chymotrypsin in the acylation of a prochiral diol in organic media by differences in the substrate solvation of the transition states leading to the product enantiomers [23]. In a later study, however, no significant influence of the energetics of substrate solvation on the enantioselectivity of lipases was found [24]. In lipases, the catalytic site is buried deep in the enzyme, thus shielding the substrate from the solvent. In the case of proteases, where the active site is open to the solvent, it makes sense that substrate solvation has a strong impact on enantioselectivity. Savile and Kazlauskas [25] explained the observation that subtilisin displayed opposite enantiopreference towards several chiral alcohols in aqueous and organic solvent by assuming different exposure to the solvent of the enzyme-bound transition state of both enantiomers. They demonstrated the effect by comparing the enantiopreference of subtilisin towards alcohols with either hydrophobic (e.g. toluyl) or hydrophilic (e.g. 4-pyridine *N*-oxide) aromatic substituents.

The reduced enantioselectivity of BS2 esterase in the conversion of hydroxynitrile esters in comparison to the analogous ethynyl derivatives suggests that the effect of substrate solvation on enantioselectivity should also be taken into account in the case of carboxyl hydrolases, especially when water can actually enter the active site.

Previous findings already showed that the enzymatic hydrolysis of arylaliphatic tertiary structures is greatly affected by subtle steric and polar effects: minute changes in the substrate structure had strong influence on activity and enantioselectivity of BS2 esterase variants [2,3]. Despite the obvious structural similarity between **1** and **2**, they seem to represent rather different substrate classes for lipases and esterases. The observed ability of enzyme-bound substrates bearing a nitrile group to form H-bonds with water molecules may explain this striking difference in selectivity and emphasizes the importance of the solvent as second coordination sphere of biocatalysts with open active sites.

4. Conclusion

The activity and selectivity of *B. subtilis* esterase BS2 and its mutants was investigated in the hydrolysis of α,α-disubstituted cyanohydrin acetates **1a**–**e**, a class of *tert*. alcohol esters. While all enzyme variants were active for these sterically very demanding substrates, only *E*-values in the range 1–4 were observed. This is in sharp contrast to the closely related class of *tert*. acetylenic alcohol acetates (**2**), which could be resolved with *E*-values above 100 (Table 3). Molecular modelling studies indicate a strong influence of water in the active site on the enantioselectivity. The drop of enantioselectivity could also be based on different steric effects as seen in a different binding mode for the slower reacting enantiomers. Taking into account that docking programs work in absence of an explicit solvent box, however, it would be difficult to explain the effect by the outcome of docking experiments in cases where distinct hydrogen bonds with flexible solvent molecules play a role. The informative value of the modelling results should therefore be limited to the finding that water can access the very open binding site of BS2 esterase and that it is able to form hydrogen bonds to enzyme-bound substrates of the type **1**, influencing both activity and/or enantioselectivity of esterases and lipases drastically. Substrates of type **2** cannot form these hydrogen bonds. The results indicate that the interaction of the substrate with the solvent – very different in the case of **1** and **2** – can be a key source for the observed difference in selectivity.

Table 3
Influence of the third substituent on activity and enantioselectivity of lipases and esterases in the hydrolysis (if not indicated otherwise) of *tert*. alcohol esters.

Enzyme	2b	2e	3	4	1b	1e	Ref.
C. rugosa lipase	n.d.	$E > 20$	n.c.[a]	n.c.	$E = 13$	n.c.	[9,19,20]
Esterase BS2 G105A	n.d.	$E > 100$	n.c.	n.c.	$E = 2.5$	$E = 1.6$	[1,2]
C. antarctica lipase A	$E = 65$[b]	n.d.	n.d.[c]	n.d.	n.c.	n.d.	[9,21]

[a] n.c.: no conversion.
[b] Transesterification in *iso*-octane.
[c] n.d.: not determined.

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