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Lipase-Mediated Conversion of Protecting Group Silyl Ethers: An Unspecific Side Reaction

Lisa M. Pick,[a] Jessica Wenzlaff,[a] Mohammad Yousefi,[b] Mehdi D. Davari,[b] and Marion B. Ansorge-Schumacher*[a]

Silyl ether protecting groups are important tools in organic synthesis, ensuring selective reactions of hydroxyl functional groups. Enantiospecific formation or cleavage could simultaneously enable the resolution of racemic mixtures and thus significantly increase the efficiency of complex synthetic pathways. Based on reports that lipases, which today are already particularly important tools in chemical synthesis, can catalyze the enantiospecific turnover of trimethylsilanol (TMS)-protected alcohols, the goal of this study was to determine the conditions under which such a catalysis occurs. Through detailed experimental and mechanistic investigation, we demonstrated that although lipases mediate the turnover of TMS-protected alcohols, this occurs independently of the known catalytic triad, as this is unable to stabilize a tetrahedral intermediate. The reaction is essentially non-specific and therefore most likely completely independent of the active site. This rules out lipases as catalysts for the resolution of racemic mixtures of alcohols through protection or deprotection with silyl groups.

Introduction

Reversible formation of silyl ethers is widely used in organic synthesis to protect hydroxyl functions from unwanted reactions. Variation of the silyl moieties enables tunable reactivity and orthogonality, and thus low complexity and high yields of synthetic reactions.[1] Based on these advantages, extension of the application of silyl ethers from mere protecting groups to the simultaneous kinetic resolution of racemic mixtures of alcohols has been an object of intensive research for many years.[2] Despite considerable advances with various chemo catalysts, however, stereospecificity is still not satisfactory. A solution might provide the use of enzyme catalysts, which often act very specifically due to the spatial layout of their active sites.

The ability of some enzymes to interact with Si–O bonds has been reported for quite some time: [3] In 1989, Michel Therisod described the transesterification of a silyl ether and butyric acid ethyl ester to ethoxy-trimethylsilane in the presence of lipases.[4] Ten years later, Cha *et al.* isolated the cathepsin-L-like silicateine-α from marine sponges and showed its ability to polymerize silica.[5] For this enzyme, Dhakili *et al.* and Sparkes *et al.* later also demonstrated hydrolysis of silyl-protected p-nitrophenol and condensation of some alcohols with triethoxysilanol (TES).[6] In 2003, the condensation of silanol to disiloxane was observed with the mammalian protease trypsin and a lipase from *Rhizopus oryzae*,[7] and in 2013 condensation of silanol with the solvent octanol was observed as a side reaction in the same set-up.[8] Nishino *et al.* showed the oligomerization of diethoxydimethylsilane by a lipid-coated lipase.[9] In all these studies, the observed catalytic activities were low but significant.

With regard to a selective protection and simultaneous stereospecific cleavage of the silyl-protected racemic alcohols in organic synthesis, the observation that lipases can catalyze the turnover of Si–O bonds was particularly relevant. Lipases (triacylglyceride hydrolases; EC 3.1.1.3) are marked by a broad substrate acceptance (including many non-natural compounds), high catalytic activity without requirement for expensive cofactors, comparably high stability under process conditions and often high enantiospecificity. Compatibility with non-aqueous media enables redirection of the natural function, *i.e.* hydrolysis, towards alternative reactions such as condensation.[10] Resulting from these advantageous features, lipases have become the most important enzymes for synthetic use including synthesis of enantiomerically pure intermediates through racemic resolution.[11]

Unfortunately, when our group first studied the enzyme-catalyzed conversion of protecting group silyl ethers with lipases, we could not detect activity.[12] The study involved conversion of primary and secondary alcohols protected with *tert*-butyldimethylsilanol (TBDMS), which is an often applied, bulky protecting group. At that time, the mechanism involved in the activity of enzymes on Si–O bonds was unclear, i.e. it was unknown, whether the observed reactions are catalytic performances of the active center of the enzymes or are merely promoted by non-specific interactions. It seems, that for the reaction to proceed, nucleophilic, basic and cationic amino acid

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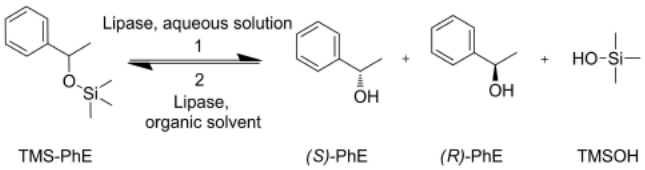
residues must be present in a certain configuration, but do not necessarily have to be located in the catalytic center of the enzyme.[13] For silicateine-α, Zhou *et al.* proposed a specific mechanism similar to the enzymatic hydrolysis of peptide bonds involving the classical triad of serine hydrolases (Ser, His, Asp).[14] For trypsin and chymotrypsin, Bassindale *et al.* suggested, that siloxane bond formation occurs at the active site, whereas cleavage is nonspecific.[7] Nishino *et al.* suspected that the lipase-mediated oligomerization they observed took place in the binding pocket of the enzyme but involved the hydroxyl functions of the lipid used for coating.[9] In principle, however, the mechanism of silicatein activity on Si–O bonds proposed by Zhou *et al.* is transferable to the active site of lipases; the condensation of silyl ethers proceeds analogously to ester formation with silanol serving as the nucleophile.[3] Therefore, we concluded from our previous results that the turnover of silyl-protected alcohols occurs in the active site of lipases, but is limited by the molecular size. This agreed with the observation, that the studied enzymes also converted bulky esters with no or very low activity.

Recently, a Brazilian working group led by Brondani has now succeeded in describing the selective turnover of trimethylsilanol- (TMS-) protected 1-phenylethanol by lipases.[15] Every of the eight lipase preparations tested in that study was able to hydrolyze the substrate to some extent using a buffered aqueous solution as reaction medium. Conversion was best with lipase B from *Pseudozyma aphidis* (formerly *Candida antarctica*), while the lipase from *Aspergillus oryzae* showed the best enantioselectivity. In contrast, only the immobilized lipase B from *P. aphidis* seemed able to catalyze silyl ether formation and in that direction accepted triethylsilanol (TES) as protecting function. Since TMS and TES are much less sterically demanding molecules, this seemed to support our theory that turnover of silyl-protected alcohols with lipases is limited by the size of the protecting group.

Prompted by this, we here set out to determine more precisely the conditions under which silyl-protected alcohols are converted by lipases, thereby laying a foundation for the development of lipases towards the synthetic use for simultaneous hydrolysis and racemic resolution of silyl-protected alcohols. We aimed for both, the influence of the reaction set-up and improved mechanistic understanding.

As model substrates, we used TMS-protected 1-phenylethanol (TMS-PhE) in aqueous solution or 1-phenylethanol and TMSOH in organic solvent, respectively (Scheme 1). For mechanistic studies, we employed a structural model of lipase B form *P. aphidis* (CalB), to which we docked TMS-PhE and the analogous ester 1-phenylethyl pivalate, respectively.



Scheme 1. Hydrolysis (1) and condensation (2), respectively, of TMS-protected 1-phenylethanol.

Results and Discussion

Hydrolysis of TMS-protected 1-phenylethanol

As a basis for our studies, we repeated the serial screening of commercial lipases for cleavage of TMS-PhE in aqueous solution described by Brondani et al.[15] We incubated a set of nine lipases in Tris-HCl buffer (pH 7.0) with the substrate and then analyzed the solutions for the hydrolysis products (*S*)- and (*R*)-1-phenylethanol (PhE), respectively. Results are illustrated in Figure 1.

In agreement with the observations of Brondani *et al.*,[15] we found a PhE content higher than in the control (lacking enzyme) with almost all lipase preparations. An exception was only PS, a lipase preparation from *Pseudomonas stutzeri*, which had not been included in the study of the Brazilian team.[15] However, compared to the results from this research group, substrate conversion in our study, as calculated from the PhE concentration after reaction, was very low. Using the same reaction conditions, Brondani et al.[15] had reported a TMS-PhE turnover of at least 28% within 24 h with all lipase preparations, whereas we could detect a maximum of 14% (with CRL, a lipase preparation from *Candida rugosa*). With CalB, which had performed best in the Brazilian team's study, we observed very low turnover. Also distinct from the reported findings, we detected a low, but increasing substrate hydrolysis over time in the absence of enzyme (3.2% within 24 h), and we did not observe significant enantiomeric excess (ee) of either the (*S*)- or

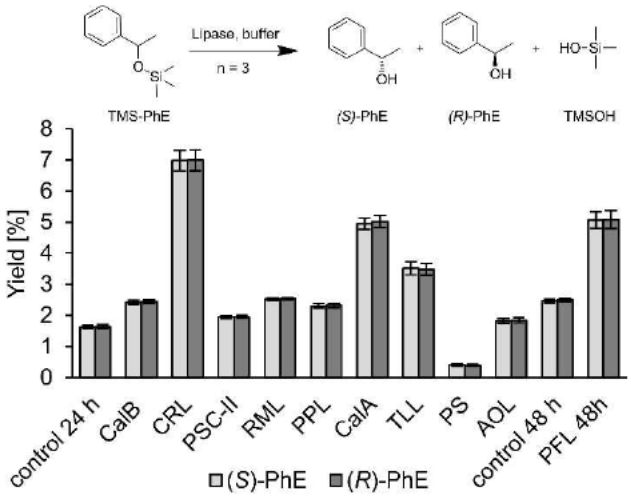


Figure 1. 1-Phenylethanol (PhE) yields from TMS-PhE hydrolysis with 20 mg mL^{-1} lipase preparations from *Pseudozyma aphidis* (CalB and CalA), *Candida rugosa* (CRL), *Pseudomonas cepacia* (PSC-II), *Rhizomucor mihei* (RML), porcine pancreas (PPL), *Thermomyces lanuginosus* (TLL), *Pseudomonas stutzeri* (PS), *Aspergillus oryzae* (AOL) and *Pseudomonas fluorescence* (PFL), respectively, in Tris-HCl (50 mmol L^{-1}, pH 7.0) after 24 hours or 48 hours (only PFL) at room temperature. The substrate concentration was 120 mmol L^{-1} TMS-PhE, Error bars indicate standard deviations from three independent experiments (n = 3). (*S*)-PhE: (*S*)-1-phenylethanol; (*R*)-PhE: (*R*)-1-phenylethanol.

(*R*)-PhE in any reaction mixture. Regarding the differences in spontaneous TMS hydrolysis, we suggest that this is due to different sensitivities of the analytics used.

The detectable PhE concentration resulting from spontaneous hydrolysis under the given reaction conditions was overall very low (Figure 2); 6 mmol L^{-1} was never exceeded. Consequently, the fraction of PhE resulting from spontaneous hydrolysis gained significance only at small substrate concentrations. In Brondani's study though, small substrate concentrations were not investigated and thus, the fraction of PhE from spontaneous hydrolysis remained inconspicuous. However, TMS ethers are known for their rapid, spontaneous hydrolysis in aqueous solutions.

The limited PhE amount obtained from spontaneous TMS-PhE hydrolysis even at high concentrations was an indication that the low solubility of this substrate in water could be crucial for conversion. Actually, when we added 10 mmol L^{-1} ß-cyclodextrin to a solution of 10 mmol L^{-1} TMS-PhE in buffer, we yielded twice as much PhE than without this solubility enhancer (Table S1, Supporting information). An even higher effect achieved the addition of 50% (v/v) isopropanol to an aqueous solution of 100 mmol L^{-1} TMS-PhE. However, addition of β-cyclodextrin or isopropanol did not enhance the PhE yield from enzyme catalysis. Likewise, addition of other solvents was mostly without effect (Figure S1A, Supporting information). An exception resulted only from the addition of ethyl acetate, with which the enzyme-coupled PhE yield increased to 83%.

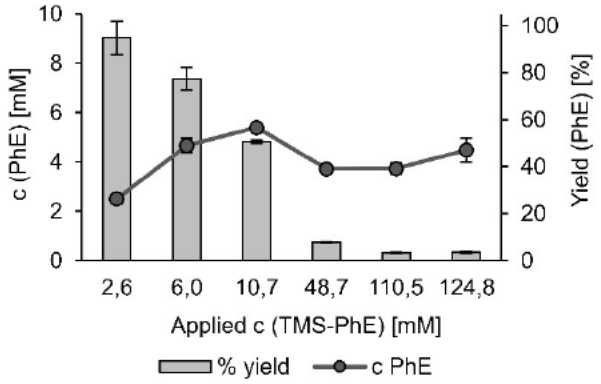


Figure 2. PhE concentration and yield resulting from TMS-PhE hydrolysis in buffer (Tris-HCl, 50 mmol L^{-1}, pH 7) after 24 h at room temperature. Error bars indicate standard deviations from three independent experiments.

Ethyl acetate is a good solvent for TMS-protected ethers and therefore often employed for chemical synthesis.[15] In pure ethyl acetate, spontaneous cleavage of TMS-PhE did not occur (Figure S1B, Supporting information). However, ethyl acetate is also a suitable substrate for lipases such as CalB.[16] Thus, we suspected that the observed high PhE formation in presence of this solvent was due to the enzyme-catalyzed hydrolysis of ethyl acetate and subsequent hydrolysis of the silyl ether by the released acid rather than TMS-PhE. Such a reaction was also recently described by Rüsch gen. Klaas *et al.*[16] Silyl ethers are generally prone to acid- or base-mediated hydrolysis; lability increases with decreasing size of the silyl moiety.[1] Therefore, ethers containing the TMS function hydrolyze already in the presence of weak acids and bases. In accordance, we had observed initially that TMS-PhE stability decreased considerably at decreasing pH (Figure S2, Supporting information). Rapidity of hydrolysis increased at lower pHs and at pH 11, but decreased at pH 8 to 10.

In fact, we found that upon addition of only 2% (v/v) ethyl acetate to the CalB-catalyzed reaction the pH dropped dramatically after a comparably short reaction time (Figure 3A), while it was constant in the absence of ethyl acetate (Figure 3B). At the same time, TMS-PhE and PhE concentrations decreased and increased, respectively. Starting from pH 8.0, an acetic acid concentration of only 0.1% (v/v) lowered the pH to 5.0 (Figure 3C). At a concentration of 1% and more, pH was as low as 3.0.

Based on this recognition, we suspected that an extraction of the reaction mixture with ethyl acetate as performed by Brondani *et al.*,[15] but not in our experiments, was at least partly

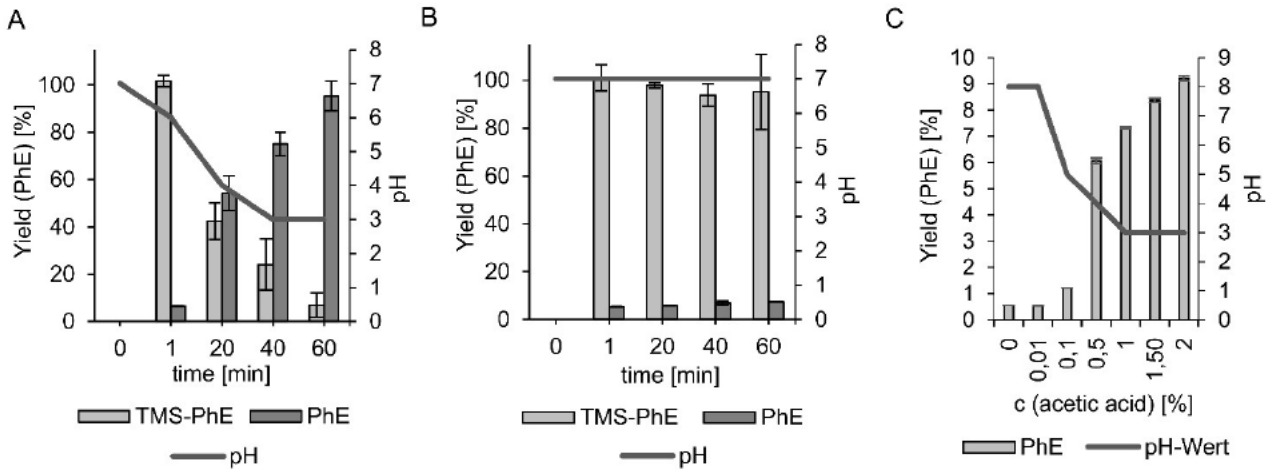


Figure 3. Ethyl acetate related pH drop and PhE release in CalB-catalyzed TMS-PhE hydrolysis. A: 10 mmol L^{-1} TMS-PhE and 20 mg mL $^{-1}$ CalB in 50 mmol L^{-1} Tris-HCl, (pH 7.0) with 2% (v/v) ethyl acetate. B: 10 mmol L^{-1} TMS-PhE and 20 mg mL $^{-1}$ CalB in 50 mmol L^{-1} Tris-HCl, (pH 7.0) without ethyl acetate. C: 10 mmol L^{-1} TMS-PhE in 50 mmol L^{-1} Tris-HCl, (pH 8.0) without enzyme at different concentrations of acetic acid. Error bars indicate standard deviations from three independent experiments.

responsible for the initially observed discrepancies in the extent of PhE formation in the two studies. We reckoned that the short exposure of ethyl acetate to the enzymes could be sufficient to initiate pH-driven hydrolysis of TMS-PhE so that considerably higher PhE concentrations were measured than actually resulted from the enzyme-catalyzed reaction. After all, the lipases were not deactivated before extraction and ethyl acetate was in a very high concentration. We observed that all investigated lipases were able to hydrolyze ethyl acetate to a varying extent (Table S2, Supporting information).

We were able to confirm our assumption, when we repeated our initial experiments with CalB and CRL using either ethyl acetate or iso-hexane for repeated extraction (Figure 4A). With CalB, we obtained more than twice as much PhE when the reaction mixture was extracted with ethyl acetate instead of iso-hexane. The acetic acid content in the GC sample was up to 5% (v/v) (Table S2, Supporting information). With CRL, the PhE content also increased considerably after extraction with ethyl acetate, yielding acetic acid up to 0.1%. Nevertheless, PhE formation with CRL was still high when iso-hexane was used for extraction.

Based on the knowledge of the strong pH effects on the hydrolysis of TMS-PhE and the obviously low buffer capacity of the media used, we checked our results described at the beginning for possible pH effects of the enzyme preparations. Actually, we found that the commercial enzyme preparations that yielded more recognizable TMS-PhE conversion (CRL and CalA) had a pH of only 6, while the commercial preparations that yielded less PhE had a higher pH (TL with pH 8). When we adjusted the pH of CRL and CalA to 7.0 before use, the TMS-PhE turnover fell into the same range as the other lipases (Figure 4B).

Together with the lack of enantiospecificity of silyl ether turnover in our study, our results strongly indicated that in our system TMS-protected 1-phenylethanol was hydrolyzed mainly nonspecifically, independent of the active site of the lipases.

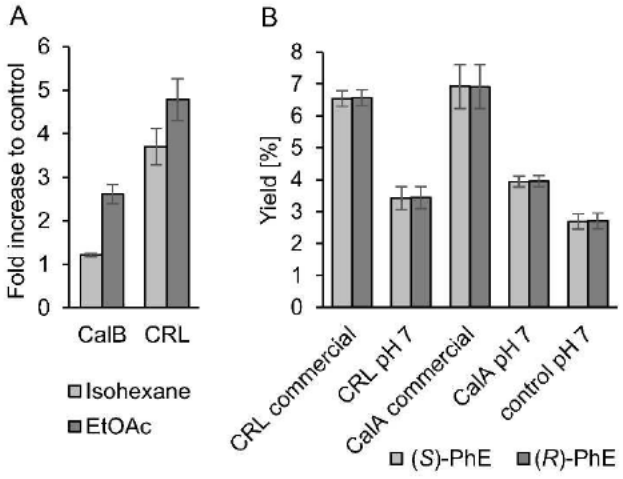


Figure 4. PhE content in samples from lipase-catalyzed TMS-PhE catalysis. A: After extraction with 2×500 µL iso-hexane or ethyl acetate, respectively. B: Involving 10 mg mL^{-1} enzyme preparations at different initial pH. Error bars indicate standard deviations from three independent experiments.

This contradicts our original theory, but is consistent with the findings of Bassindale *et al.*, who investigated the hydrolysis of trimethylethoxysilane (TMS-OEt) mediated by the mechanistically related serine-protease trypsin.[7] It also fits with our further observation that TMS-PhE was also hydrolyzed in the presence of bovine serum albumin (BSA), a protein lacking a catalytic center (Figure S3, Supporting information). At physiologic pH this protein is a weak acid with a negative net molecular charge (isoelectric point of BSA is 4,5).[17] It is found in literature that high concentrations of BSA decrease the buffering capacity of blood.[18] Possibly, this indicates that protein acidity favors the hydrolysis of TMS-PhE.

Condensation of TMS and 1-phenylethanol

In a study on the trypsin-mediated condensation of TMS or phenyldimethylsilanol (PDMS), respectively, and 1-octanol, Abbate *et al.*[8] suggested that the condensation of silyl ether bonds was catalyzed at the enzyme's active site, although hydrolysis was probably not. Similar deductions were made for lysozyme and a lipase from *Rhizopus oryzae*. Hence, we also examined TMS-PhE formation with CalB and included the condensation of TMS with 1-octanol and phenol, respectively. With all three alcohols, we obtained silyl ethers to a significant extent (Figure 5). Notably, however, phenol is not a regular substrate of CalB and therefore should not be converted at the active site.[19] In addition, PhE was used without enantiospecifically (data not shown), although CalB is known to distinctly react on the (*R*)-enantiomer.[20]

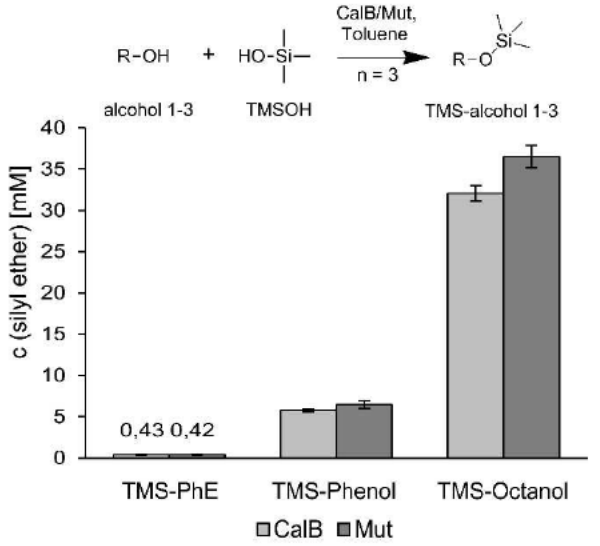


Figure 5. Formation of TMS-ether from 100 mmol L^{-1} trimethylsilanol and 100 mmol L^{-1} 1-phenylethanol (1), phenol (2) or 1-octanol (3), respectively, in dry toluene in presence of 100 mg mL^{-1} immobilized active lipase B from *Pseudozyma aphidis* (CalB) or a deactivated mutant CalBS105A (Mut). Both enzymes were produced recombinantly in *E.coli* and immobilized on Accurel MP1001 (enzyme loading: 1,7–2% w/w), the reaction was run at room temperature over 14 days. Control reactions with only Accurel MP1001 did not show relevant product formation (data not shown). Error bars indicate standard deviations from three independent experiments. R = corresponding alcohol 1–3.

These findings further support our theory that the active site of the lipase was not involved also in the condensation of silyl ethers. Further confirmation of this assumption was provided by the observation that two variants of CalB, which no longer exhibited catalytic activity due to replacing the serine (S105A) or both serine and histidine (S105A/H224A) of the catalytic triad,[20b,21] favoured the condensation of silyl ethers in the same way as the native enzyme (Figure S5, Supporting information). On the other hand, replacement of the His_6-tag of recombinantly generated CalB or its variants with a Strep-tag reduced TMS-PhE formation to almost nothing (Figure S4, Supporting Information). This effect had also been observed in the context of silicatein-α[6b] and could indicate an involvement of the His-tag in silyl ether condensation.

Interaction between TMS-protected 1-phenylethanol and lipase

To finally evaluate the selective impact of lipases on cleavage and formation of TMS-PhE, we performed a computational study on the binding of TMS-PhE at the active site of CalB, because reliable structural information was available for this enzyme.

In preparatory experiments, we demonstrated that under the conditions investigated here, CalB is able to hydrolyze 1-phenylethyl pivalate (Figure S5, Supporting information), which is the structurally nearest possible ester analogue of TMS-PhE. As expected, the activity was distinctly (*R*)-specific. In accordance, covalent mechanism-based docking of 1-phenylethyl pivalate in the active site of a structural model of the enzyme showed a catalytically competent pose for the (*R*)-, but not the (*S*)-form of this substrate with a binding energy of -3.85 kcal mol^{-1}. As proposed for the catalytic mechanism of lipase, a hydrogen bond was built between protonated His224 and the oxygen of the ester bond in the first tetrahedral intermediate, which plays an important role in the next step to release the alcohol (Figure 6).[22] In addition, the hydrogen bond between the anion oxygen of the carbonyl group and the oxyanion hole residues (Thr40, Gln106, and Gln157) was formed. This hydrogen bond is reported to play an important role to stabilize the first tetrahedral intermediate of the ester. Notably, only the (*R*)-enantiomer of the carbonyl ester substrate can form the relevant hydrogen bond interaction, while no catalytically competent docking pose was found for the (*S*)-enantiomer of this substrate.

Based on the fitting of 1-phenylethyl pivalate in the binding pocket of CalB, we assume that also the accommodation of TMS-PhE should be possible sterically. Non-covalent docking supported this theory. However, covalent docking analogous to the pivalate showed that no catalytically competent binding pose can be identified either for the (*R*)- or for the (*S*)-form. Previous predictive docking studies demonstrated that Distance 1 (Figure S6) plays a vital role in lipase-catalyzed hydrolysis.[22] This distance criteria is fulfilled for the carbonyl based substrate, while it was not identified for silyl based substrates. These results correlate with our experimental finding of the inability of lipase to hydrolyze silyl based substrates as compared to the carbonyl ester substrate.

To understand the energy profile of the rate-determining step in the reaction we applied semi-empirical quantum mechanics calculation. It is known that the formation of tetrahedral intermediate 1 (Figure S6) is the rate-determining step for serine hydrolases. [21,23] Our semi-empirical calculation (Figure S8) showed that there is a 23 kcal mol^{-1} energy barrier for the carbonyl substrate to form the tetrahedral intermediate 1. In contrast, this value for the silyl-based substrate is much higher (35.5 kcal mol^{-1}). Although there is no barrier to produce alcohol from the tetrahedral intermediate 1 of the carbonyl based substrate ($\Delta Hf = -4.7$ kcal mol^{-1}), the methyl group in the silyl-based substrate acts as a protection group and prevents alcohol hydrolysis through steric hindrance of the

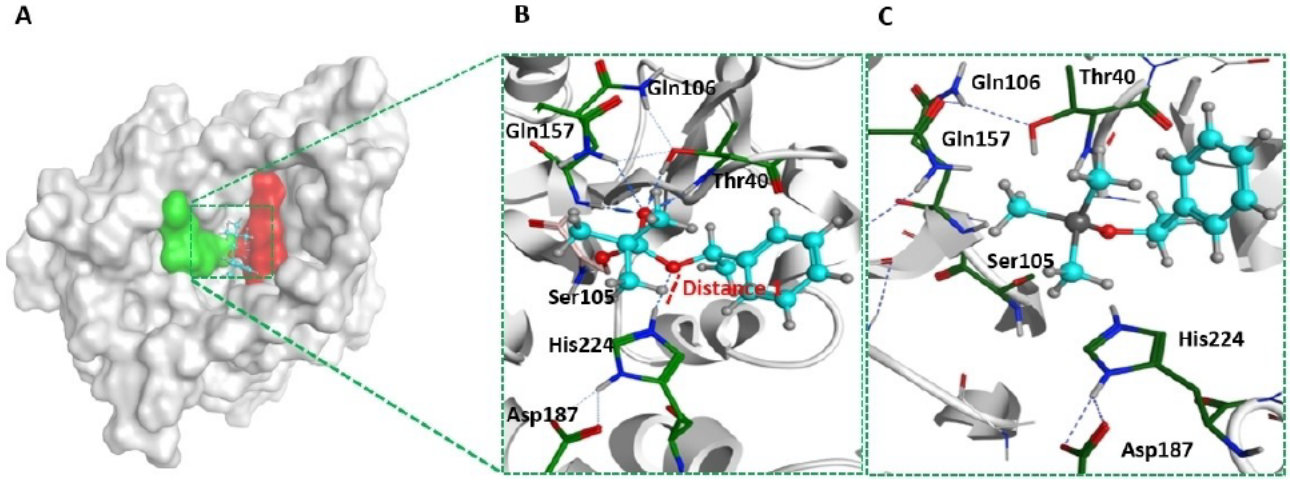


Figure 6. Molecular docking of substrates to lipase B from *P. aphidis* (CalB). A: Molecular surface of CalB. Oxyanion hole and catalytic triad residues are highlighted in red and green color, respectively B: Covalent catalytically competent binding pose of 1-phenylethyl-pivalate in the active site, C: Non-covalent docking of silyl ether substrate (catalytically competent binding pose was found). All interacting amino acid residues (Thr40, Gln106, Gln157, Ser105, and His224) are shown in stick representation and green color; covalently linked Ser105 side chain is shown in brown color. Substrate is shown in ball and stick and cyan color. The hydrogen bond interaction between His224 and oxygen ester is shown as Distance 1 (2.19 Å).

protonation of the ether oxygen by the methyl group of the silyl-based substrate. This means that even if the silyl-based substrate binds to the CalB active site, it might inhibit enzyme activity.

Conclusions

In our study, we confirmed that lipases mediate the hydrolysis and formation of TMS-protected 1-phenylethanol. Thus, in principle, they can be considered as a tool for protecting group chemistry. However, the activity due to the presence of the enzymes was found to be low. A considerable part of the observed conversion of TMS-PhE resulted from spontaneous hydrolysis, which is already favored by slight decreases in pH. If the pH is lowered in the course of sample processing, this leads to a false positive activity.

Surprisingly, and contrary to reports in the literature,[15] not only the spontaneous hydrolysis of TMS-PhE proceeds without enantiospecificity, but also the lipase-mediated portion. Given the otherwise usually high enantiospecificity of lipases, this strongly suggests that silyl ether cleavage does not proceed in the active site of lipases. The same seems to be true for the condensation reaction, which is also mediated by CalB without an intact catalytic triad.

Indeed, mechanistic studies at the active site of CalB show that while TMS-PhE can be incorporated into the active site, a positioning that allows the mechanism of hydrolysis proposed by Zhou *et al.*[14] to proceed is not possible. However, even if such positioning could be achieved, e.g. by site-directed mutation, the catalytic triad of lipases would almost certainly not catalyze silyl ether cleavage because the substitution of the silicon atom in the silyl function does not allow the formation of a stable tetrahedral intermediate. With regard to the strong conservation of the reaction mechanism in lipases,[24] this probably applies to the whole enzyme group. Thus, a promiscuous mechanism aiding silyl ether conversion independent of the active site must be assumed. This might fall into the same category as the known effects of proteins without discrete enzymatic activity on similar base-catalyzed reactions, such as the Kemp elimination of benzisoxazole,[25] β-elimination of umbelliferone ethers[26] and the Morita-Baylis-Hillman reaction for coupling of cyclohexanone and o-nitrobenzaldehyde with BSA.[3,27] Unfortunately, due to their unspecificity, these activities are hardly advantageous over chemo-catalytic approaches.

Our observation that TMS-PhE formation by recombinant CalB decreases significantly when the His-tag inserted for purification is replaced by a Strep-tag indicates the involvement of these surface histidines in the reaction. Indeed, a similar effect has already been observed for recombinant silicatein-α.[6b] Meanwhile, for this enzyme, Sparkes proposed a new reaction mechanism that assumes the involvement of more than one histidine residue in ether hydrolysis, even in the active site, while serine is not involved.[28] Indeed, the common use of imidazole as an auxiliary base in the chemical synthesis of silyl ethers[29] also suggests that histidines are the main catalytically active residues. However, such a mechanism would imply that classical serine hydrolases such as the lipases studied here are in principle not suitable candidates for specific formation and cleavage of silyl ethers with simultaneous resolution of racemic silyl-protected alcohols. Suitable catalysts must be found in other enzyme families.

Experimental Section

Commercial enzymes and chemicals

CalB was a kind gift of c-Lecta (Germany). CRL, PSC-II, RML, PPL, AOL, TLL, PFL, and BSA were purchased from Sigma Aldrich (Germany). PS, also known as Lipase TL, was purchased from Meito Sangyo (Japan). All commercial enzymes were used without further modification, if not stated otherwise. Chemicals for the synthesis of substrates and ready-to-use substrates, buffers, and media were purchased from Sigma Aldrich (Germany), except TMSOH, which was bought from Acros (Germany). Solvents were purchased from Roth (Germany).

Recombinant enzymes

The pET22b-CalB expression vector was a kind gift from Dr. Linda Otten (TU Delft). Expression, purification, and immobilization of CalB and its variant were performed as previously described.[30] CalB variants were generated by PCR using site-specific primers.

Synthesis of silyl ethers

Under nitrogen atmosphere, 10 mmol alcohol and 15 mmol imidazole were stirred in ice cold dry DMF sitting in an ice bath. 12 mmol silyl chloride was added dropwise. The solution was stirred for 20 h slowly warming up to room temperature. The reaction was stopped by quenching with ice cold water and extracted with ethyl acetate (3x). The organic phase was further washed with water before drying over $MgSO_4$, filtering and evaporating the solvent. The crude extract was further purified by column chromatography (silica gel, isohexane/ethyl acetate 10:1). ^{1}H-NMR data are given in the Supporting information.

Enzyme assays

All enzyme assays were performed in glass vials. For hydrolysis assays, 10 mg enzyme was dissolved in 50 mmol L^{-1} buffer as stated, substrate and further additives were added as stated and the reaction mixture was shaken at 400 rpm at room temperature. For analysis, the whole reaction mixture was extracted using isohexane if not stated otherwise. Condensation was performed in dry toluol using 100 mmol L^{-1} substrates and immobilized CalB (10 mg Accurel MP1001-CalB/Mut with 1,5-2% w/w enzyme loading). Lipase activity was checked using 4-nitrophenol acetate (dissolved in EtOH, final conc. 0,5 mmol L^{-1}) in 50 mmol L^{-1} Tris-HCl pH 8. For hydrolysis assays, the pH of the reaction mixtures was determined with pH test stripes (Macherey-Nagel, Germany). Analysis was performed by gas chromatography on a Shimadzu GC-2010 Pro equipped with an AOC-auto sampler, FID and a chiral HYDRODEX GAMMA-DiMOM column (Macherey-Nagel, Germany). Nitrogen was used as carrier gas with a pre-column pressure of 100 kPa. 2 µl of sample were injected with a split of 1:30. The temperature program was set at 110 °C (14 min) for detection of silyl-bases substrates and their corresponding alcohols and 110 °C (11 min)→150 °C (1 min) at 20 °C/min for 1-phenylethyl pivalate. Typical retention times are; TMS-PhE (4.5 min), S-(9,3 min) and R-PhE (10 min), acetic acid

(2 min), TMS-Octanol (4,5 min), 1-Octanol (5,8 min), TMS-Phenol (3,3 min) and phenol (10,1 min).

Molecular modeling

Non-covalent and covalent molecular docking of substrates was performed using MOE (Molecular Operating Environment) 2020.[31] The X-ray crystallography structure of Cal B (PDB ID: 5GV5, Resolution 2.89 Å;[32]) was used as starting structure. The crystal structure has eight chains. Because CalB was shown to be a monomer in solution and the active site is located far from the interface, only one chain (A) was used for docking and the co-crystalized ligand was removed from the pdb file. Substrates and receptor preparation and energy minimization was performed with Quickprep in MOE. The protonation states of His224 and Ser105 residues were assigned according to the pKa calculation using PROPKA3 server at pH 7 and the hydrogen bond scheme in the first tetrahedral intermediate (TI1) of the catalytic reaction (NE2 atom of catalytic residue His224 was protonated and the oxygen atom in the side chain of Ser105 was deprotonated).[22,33] GBV/WSA dG scoring function was used for scoring of docking poses. In our mechanism-based covalent docking of substrates, based on the proposed structure for first tetrahedral intermediate (Figure S6), a covalent bond was formed between the oxygen atom at the side chain of Ser105 and the carbonyl carbon (Distance 2) of 1-phenylethyl-2,2-dimethylpropanoate and the Si atom of TMS, TES, TBDMS, TIPS, TBDPS, and TPS, respectively. In order to reproduce the first tetrahedral intermediate (TI1) based on the catalytic mechanism[22] and to select only the catalytically competent docking poses, pharmacophore features were applied to impose the catalytic distance criteria. The key pharmacophore features such as the hydrogen, aromatic ring, and AtomQ with average radius 1.2, 1.2, and 0.3 Å were generated, respectively. These pharmacophore features fulfill the catalytically required distance criteria in TI1 for hydrogen interaction between His224 and oxygen ester (Distance 1) and also hydrogen bond interaction between oxygen in the carbonyl group of the substrate with Thr40, and Gln106 (Figure S8). 150 induced fit (flexible) docking runs were performed using the standard settings within the MOE. The validation of the docking protocol was performed by re-docking the co-crystalized ligand (phosphonate inhibitor) to the CalB, using the same parameters, which resulted in a high superposition and low RMSD (root mean square deviation) value of 1.33 Å between the docked and co-crystalized ligand due to small rearrangements of some atoms.

Quantum chemical calculations

To study the energy profile of CalB catalysed reaction, semi-empirical quantum chemical calculation was performed by using PM7 method in the Molecule Orbital PACkage (MOPAC) 2016 (version 19.266 L).[34] In order to decrease the computational time, most of the amino acid residues in the active site (including the catalytic triad and oxyanion hole of enzyme, see list of residues in Table S3) were selected for quantum chemical calculations). In order to avoid the unexpected movement of backbone atoms, they were fixed and the side chain atoms were considered flexible. The Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm was used for optimization, and the rest of the parameters were kept as default in MOPAC calculations. The potential energy scan through grid calculation was performed with a step size of ±0.2 and ±0.1 Å, respectively (i.e., 2D scan of deprotonation of Ser105 through NE2 atom of catalytic residue His224 and nucleophilic attack of O of Ser105 to carbonyl carbon of ester substrate, or silicon for silyl-based substrate). The final heat of formation (ΔHf) of the system was calculated for each step.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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