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Desymmetrization of cbz-serinol catalyzed by crude pig pancreatic lipase reveals action of lipases with opposite enantioselectivity

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ABSTRACT

Lipase preparations from pig pancreas are widely used in industrial production of fine chemicals and pharmaceuticals. In our attempts to synthesize enantiopure building blocks for production of iminocyclitols, we used a crude preparation of pig pancreatic lipase in acetylation of carboxybenzyl-2-amino-1,3-propanediol (cbz-serinol) with vinyl acetate in tetrahydrofuran. The enantiomeric excess of the product monoester changed from (*2S*)-monoacetate to (*2R*)-monoacetate after prolonged reaction. By using vinyl acetate both as solvent and acyl donor only the (*2R*)-monoacetate was obtained. Purified enzyme preparation gave only the (*2S*)-monoacetate. It is likely that the crude enzyme preparation contains lipases with opposite stereoselectivity, acting at different rates in the desymmetrization reaction. The same enzyme preparation was used in hydrolysis of the diacetylated propanediol in phosphate buffer and acetonitrile as co-solvent. After 24 h the (*2S*)-monoacetate was obtained in high enantiomeric excess. Our results show that monitoring the progress of biocatalyzed reactions are of utmost importance, in particular when crude enzyme preparations are used as catalysts.

1. Introduction

Hydrolytic enzymes have for many years been used with great success to obtain enantiopure compounds, both in hydrolysis of esters and transesterification reactions in organic media [1,2]. Kinetic resolution of racemic mixtures is a very common method, but desymmetrization of a *meso* or prochiral substrate is also widely used. During kinetic resolution, the enantiomeric excess of the product fraction (ee_p) decreases as the reaction progresses, but the enantiomeric excess of the remaining substrate (ee_s) increases and will always reach 100% at some conversion higher than 50%. The important parameter for a kinetic resolution process is the *E*-value, which for practical purposes is the ratio of the rate constants for reaction with the two enantiomeric substrates. For desymmetrization the important parameter is the enantiomeric excess (ee) of the product. The *E*-value in kinetic resolutions and the ee in desymmetrizations are parameters considered to be constant throughout the reaction [1]. However, we have previously shown that in kinetic resolutions catalyzed by lipase B *from Candida antarctica* (CALB) the *E*-value decreased by decreasing conversion in transesterification of a series of secondary alcohols, while the *E*-value increased by increasing conversion in hydrolysis of the corresponding butanoates. This shows that monitoring the reactions is important in all biocatalyzed reactions. We found that the changing concentrations of the (*R*)-alcohols in the medium were causing this effect by a possible allosteric inhibition (activation) of the lipase [3,4]. We have also shown that acetaldehyde from the acyl donor was not causing any effect on CALB [5].

Lipase preparations from pig (porcine) pancreas (PPL) are used in industrial production of fine chemicals and pharmaceuticals (DSM, Lonza, Sigma–Aldrich, etc.), f. inst. glycidol and cyclopropanediol [6,7]. Enzyme extracts from pig pancreas (Creon®, Solvay Pharmaceuticals, Switzerland) have also been used for many years as aid for digestion of patients suffering from pancreas dysfunctions and malnutrition, among them 70,000 Cystic Fibrosis patients worldwide (http://www.cff.org/AboutCF).

Lipase preparations of animal origin tend to consist of a collection of enzymes which catalyze fat hydrolysis. Although the crude preparation PPL Type II from Sigma–Aldrich has been purified, protease and amylase activity are still observed. Monitoring the progress of crude PPL catalyzed reactions is therefore important in order to verify the action of competing enzymes. Fractionation of crude PPL Type II from Sigma–Aldrich has been performed [7–9]. Three lipases were identified as 25-kDa, 33-kDa and the most

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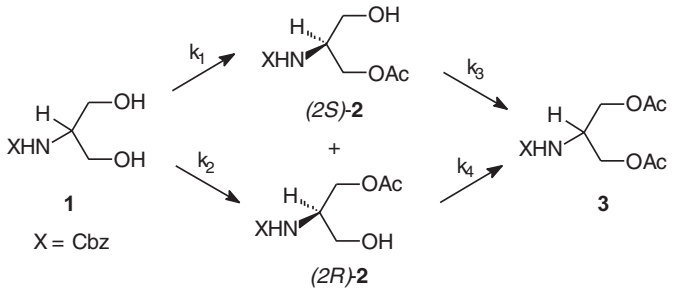

Scheme 1. Acetylation of prochiral carboxybenzyl-2-amino-1,3-propanediol (**1**) with vinyl acetate catalyzed by pig pancreatic lipase (PPL).

abundant PPL (52-kDa) [9]. These fractions have been compared in the resolution in aqueous media of different chiral compounds that are precursors of drugs [10]. In immobilized forms, the PPL enzyme fractions have been tested as catalysts for kinetic resolution of secondary substrates, f. inst. ethyl 2-hydroxy-4-phenylbutanoate [9]. The (*2S*)-enantiomer is the faster reacting enantiomer in hydrolysis reactions catalyzed by all three enzymes. However, the *E*-values are low (7–10), indicating that this is not a good substrate for the lipases in crude PPL. Both (*R*)- and (*S*)-monoesters have been made from PPL catalyzed hydrolysis of different prochiral diesters, however, the configuration will depend of the substrate structure [11,12]. Based on the actual kinetic mechanism Van Tol et al. have made a kinetic model system for kinetic resolutions catalyzed by crude PPL [13]. In inhibition experiments of PPL Sigma Type VI-S (purified enzyme) a proteinaceous inhibitor from wheat flour caused non-competitive inhibition. This indicates that this enzyme may have a mechanism of action different from that of the reported soybean-derived lipase inhibitors which caused competitive inhibition [14].

Enantiopure derivatives of 1,3-propanediols are used as precursors for bioactive compounds such as iminocyclitols (glycosidase-inhibitors), phospholipids, platelet activating factor and rennin inhibitors [15]. Desymmetrization of prochiral 1,3-propanediols and diacetates in the presence of lipases has become a practical approach for preparation of chiral compounds due to high specificity and reproducibility [16]. Monoacetates are in principle formed at unequal rates, and also react further at unequal rates in a double step process (see Scheme 1). The first step is desymmetrization, the second, kinetic resolution. It is anticipated that if $k_1 > k_2$ then $k_4 > k_3$, and moreover, that the ratio k_1/k_2 is constant throughout the reaction [1].

2. Experimental

2.1. Enzyme catalysts

Preparations of pig pancreatic lipase (PPL, EC 3.1.1.3 and CAS 9001-62-1) were purchased from Sigma–Aldrich: crude PPL L3126 Type II (Steapsin), Lot 096K0747 and Lot 096K0721. Analyses (by Sigma–Aldrich, Buchs, Switzerland) of Lot 096K0747 show 38% protein, 358 U/mg protein (olive oil, 30 min incubation)/50 U/mg protein (triacetin), amylase activity: 147 U/mg, general protease activity: 2.5×10^{-4} U/mg, α-chymotrypsin activity: 7.2×10^{-2}. Pure PPL L0382, Type VI-S, lyophilized powder, ≥20,000 U/mg, 50% protein. Immobilized Lipase B from *Candida antarctica* (Novozym 435, 10 U/mg) was a gift from Novozymes AS, (Bagsværd, Denmark). Unit definition: One unit will hydrolyze 1.0 μequiv. of fatty acid from olive oil in 1 h at pH 7.7 at 37 °C. Trypsin (Type II crude) from pig pancreas T-8128, was purchased from Sigma–Aldrich, Lot 96F-06891. Trypsin activity: 1740 U/mg (BAEE units) and chymotrypsin activity: 1370 U/mg (ATEE units). Esterase from hog liver (pig liver esterase) was purchased from Böhringer Mannheim, Lot 10315523-22, activity not given.

2.2. Chemicals

Analytical grade chemicals were purchased from Sigma–Aldrich. Solvents were from VWR International. Purification and separation of products were performed using VersaFlashTM from Supelco, Sigma–Aldrich, column VersaPakTM (silica, spherical 23 × 110 mm, 23 g), Cat. No. 97758-U, max. pressure 60 psi, eluent pentane:EtOAc, 4:1. Except for entry C (see Table 1) the THF used in enzymatic reactions was dried over molecular sieve. The dry THF used for reaction C was distilled under N_2 from Na/benzophenone.

2.3. Chromatographic analyses

Chiral HPLC analyses in entry C were performed with two instruments; a Varian HPLC coupled to a Varian 2550 UV detector with a Varian Prostar 4000 autosampler and an Agilent 1100 Series: G1379A Degasser, G1311A QuatPump with G1313A coupled to a Bruker UV detector and an ALS autoinjector. Column Daicel Chiralcel OD-R (i.d. 4.6 mm, 25 cm, particle size 10 μm). Eluent A: H_2O (40%) + CH_3CN (59.9%) + TFA (0.1%). Eluent B: H_2O + TFA (0.1%). Gradient elution: 65% A + 35% B to 95% A + 5% B over 25.72 min (flow 1.0 mL min^{-1}). t_R **1**: 5.2 (5.4) min, t_R (*2S*)-**2**: 7.8 (8.1) min, t_R (*2R*)-**2**: 10.9 (11.9) min, and t_R **3** (diacetate) 20.8 min. Chromatograms were recorded and analyzed using Varian Star Chromatography Workstation 6.0 software (chromatograms in Fig. 1). Reaction mixtures from entries A and C–G were analyzed by GLC using a Varian 3380 Gas Chromatograph with a Varian CP-8410 autoinjector and Varian Star Chromatography Workstation 6.0 software, CP-Chirasil DEX column (i.d. 0.32 mm, 25 m, film density 0.25 μm), carrier gas H_2 (5.0), injection temp. 250 °C, FID temp. 270 °C, column pressure 7.5 psi, split flow 60 mL min^{-1}. Temp. progr. 80–92 °C/30 °C min^{-1}, 92–98 °C/3 °C min^{-1}(2), 98–104 °C/3 °C min^{-1}, 104–140 °C/30 °C min^{-1}, 140–200 °C/3 °C min^{-1}. t_R diol **1**: 11.8 min, monoacetates: t_R (*2S*)-**2** 17.3 min, t_R (*2R*)-**2**: 18.8 min, monobutanoates: t_R (*2S*)-**4**:17.4 min, t_R (*2R*)-**4**: 18.7 min and t_R diacetate **3**: 33.1 min. $R_S > 1.5$ for monoester enantiomers in all chromatographic analyses, (*2R*)-**2** was identified in accordance with Choi and Borch [17]. Thin layer chromatography was performed using Merck Silica Gel 60 F_{254} TLC plates using UV light at 312 nm.

2.4. Immobilization of crude PPL

Powdered polypropylene (Accurel MP1000, 1.5 g) was added to EtOH (96%, 3 mL). PPL (L 3126, Type II (crude) Steapsin, 1.5 g) was suspended in phosphate buffer (20 mM, 30 mL). The two solutions were mixed and stirred for 22.5 h at RT. The immobilized enzyme was filtered off, washed with dist. H_2O and lyophilized for 16 h.

2.5. Enzymatic acetylation acetylation of *1*

See Table 1, entries A–G for amounts. Substrate **1** (0.18–1.8 mmol) and vinyl acetate or vinyl butanoate (20 μL–0.72 mL) were mixed in solvent, (2–10 mL). The reactions were initiated by addition of enzyme (0.0012–0.252 g). The reactions were stirred at 200 rpm under N_2 at RT in a closed flask. Samples (50 μL) were withdrawn at various intervals before halting the reactions by filtering off the enzyme catalyst with addition of EtOAc (entries A and B) or CH_2Cl_2 (entries C–G). All reactions were performed in duplicate. In entry B the reaction mixtures were analyzed by chiral HPLC, in entries A and C–G by

Table 1
Enzymatic esterification of **1**, entries A–G. Hydrolysis of diacetate (**3**) entry H. Entries B and H were analyzed by chiral HPLC, entries A and C–G by chiral GLC. Duplicates not shown. Duplicates with PPL Type II Lot 096K0721 gave similar results as in entry D. Yield in % are shown for isolated products.

Entry	Substr./amount	Enzyme	Enz. amount	Solvent	VA: vinyl acetate VB: vinyl butanoate	Rx. time (h)	Monoester in excess at rx. end	Yield %
AFigs. 2 and 3	**1**/0.0811 g, 0.360 mmol	Untreated PPL L3126, Sigma Type II Steapsin Lot 096K0747 358 U/mg	180 mg/mmol substr., 0.0654 g	THF, 2 mL	VA: 35 μL, 0.376 mmol (1.0× exc.)	3	(*2R*)-**2**, 70% ee	
B Fig. 1	**1**/0.4086 g, 1.814 mmol	Immob. PPL L3126, Sigma Type II Steapsin Lot 096K0747 358 U/mg	140 mg/mmol substr., 0.252 g	THF, 10 mL	VA: 0.72 mL, 7.73 mmol (4.3× exc.)	9	(*2R*)-**2**, 98% ee	
C	**1**/0.0563 g, 0.250 mmol	Untreated PPL L3126, Sigma Type II Steapsin Lot 096K0747 358 U/mg	300 mg/mmol substr., 0.0755 g	Vinyl acetate, 5 mL, 53.7 mmol		1.5	(*2R*)-**2**, 98% ee $[\alpha]^{20}_D$ (EtOAc, c 0.797): −7.4. $[\alpha]^{20}_D$ ($CHCl_3$, c 1.0): +1.84	62
D	**1**/0.0407 g, 0.181 mmol	Untreated PPL L3126, Sigma Type II Steapsin Lot 096K0747 358 U/mg	360 mg/mmol substr., 0.0651 g	THF, 1 mL	VA: 20 μL, 0.215 mmol (1.2× exc.)	17	(*2R*)-**2**, 98% ee	
E	**1**/0.0816 g, 0.362 mmol	Untreated Sigma Type VI-S, lyophilized powder 20,000 U/mg	33 mg (pure enz)/mmol substr., 0.0012 g	THF, 2 mL	VA: 40 μL, 0.432 mmol (1.2× exc.)	6	(*2S*)-**2**, 100% ee	
F	**1**/0.0816 g, 0.362 mmol	Untreated crude trypsin/chymotrypsin from pig pancreas Sigma Type II Lot 96F-06891 Trypsin activity: 1740 U/mg (BAEE units) and chymotrypsin activity: 1370 U/mg (ATEE units)	180 mg/mmol substr., 0.065 g	THF, 2 mL	VB: 40 μL, 0.432 mmol	19	(*2S*)-**4**, 100% ee	
G	**1**/0.0816 g, 0.362 mmol	Untreated pig liver esterase Böhringer Mannheim Lot 10315523-22	180 mg/mmol substr., 0.065 g	THF, 2 mL	VB: 40 μL, 0.432 mmol	19	(*2S*)-**4**, 100% ee	
H	**3**/0.830 g, 3.69 mmol Substrate	Untreated PPL L3126, Sigma Type II Steapsin Lot 096K0747 358 U/mg	1020 mg/mmol substr., 2.8 g	Phosphate-buffer, CH_3CN		24	(*2S*)-**2**, 91% ee $[\alpha]^{20}_D$ ($CHCl_3$, c 1.0): −1.82	60

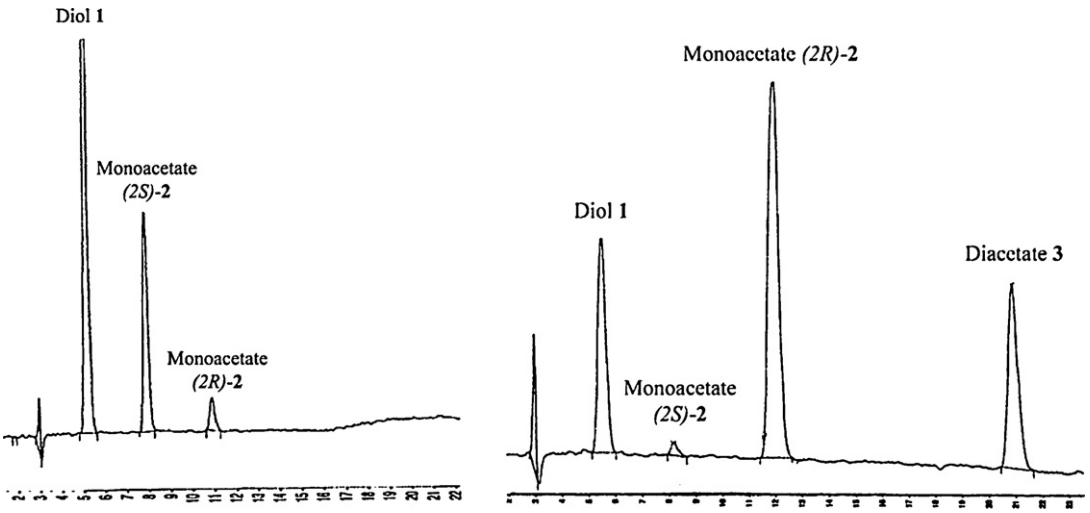

Fig. 1. Left: HPLC chromatogram of acetylation of **1** with crude PPL and vinyl acetate in THF after 30 min, see Table 1, entry B, t_R **1**: 5.2 min, t_R (*2S*)-**2**: 7.8 min, t_R (*2R*)-**2**: 10.9 min. Right: HPLC chromatogram of the same reaction after 3 days t_R **1**: 5.4 min, t_R (*2S*)-**2**: 8.1 min, t_R (*2R*)-**2**: 11.9 min and t_R **3**: 20.8 min. After 9 days the ee of (*2R*)-**2** was 98%.

chiral GLC. The HPLC samples were diluted with H_2O:CH_3CN, 1:1 before injection.

2.6. Enzymatic hydrolysis of **3**

See Table 1, entry H. The anion exchange material Macro-Prep High Q support was filtered with dist. H_2O (20 mL), phosphate buffer (50 mL, 2 mM, pH 7) then washed with dist. H_2O until the filtrate reached pH 6.7. Diacetylated cbz-serinol (**3**) (0.830 g, 3.69 mmol) was dissolved in CH_3CN (110 mL) and dist H_2O (100 mL). Then crude PPL (2.8 g, 1020 mg enzyme/mmol substrate) and the anion exchange material (100 mg) were added. The reaction mixture was stirred on water bath (25 °C) for 24 h. The enzyme and anion exchange material was filtered off and the solvent was removed under reduced pressure. EtOAc (100 mL) was added and the reaction mixture was extracted with H_2O/NaCl-solution (10/90, 2 × 50 mL), then dried over Na_2SO_4 before the solvent was removed under reduced pressure. The product ((*2S*)-**2**) was adsorbed on silica before flash chromatography.

2.7. Characterization of compounds

Optical rotations were determined using a Perkin-Elmer 243 B instrument (Wales, UK), concentrations in g/100 mL. ^{1}H and ^{13}C NMR spectra (Bruker Avance DPX instruments 300/75 MHz and 400/100 MHz) were obtained from solutions of $CDCl_3$ or DMSO-d_6, chemical shifts are in parts per million and rel. to TMS. Coupling constants are in Hertz (Hz). The spectra were processed using the software XWIN-NMR 3.5 from Bruker Biospin. Mass spectra were recorded using a MAT 95 XL (ThermoQuest Finnigan, San Jose, CA, USA) with EI probe (DIP). Melting points were obtained from a capillary tube melting point apparatus from Sanyo Gallenkamp (USA). Characterizations of compounds were in accordance with earlier reported data [17–19].

2.7.1. *N*-Benzyloxycarbonyl-2-aminopropane-1,3-diol (**1**)

Mp 110.3–112.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.33 (br s, 2H, OH) 3.74–3.95 (m, 5H), 5.14 (s, 2H), 5.50 (br s, 1H), 7.32–7.55 (m, 5H). ^{13}C (100 MHz, DMSO-d_6): δ 55.4 (1C, CH), 60.9 (1C, CH_2) 65.5 (1C, CH_2), 128.1 (3C), 128.7 (2C), 138.5 (1C), 156.3 (1C, —C—). TLC: R_f 0.8 (EtOAc: Hexane, 2:1). MS (EI) (*m*/*z*) $M^{\bullet+}$ 224.8, 193.9, 107.9, 90.9 (100%), 78.9, 64.8, 80.8, 42.9.

2.7.2. *N*-Benzyloxycarbonyl-2-aminopropane-1,3-diyl diacetate (**3**)

The diacetate **3** was synthesized by reacting **1** (0.9013 g) in vinyl acetate (5 mL) with lipase B from *Candida antarctica* (CALB, Novozym 435) (0.0401 g, 10 mg/mmol substrate) for 23 h. The diester was purified by flash chromatography using EtOAc:hexane, 2:1 to 100% purity (GLC) in 90% yield. Mp 47.1–49.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.07 (s, 6H), 4.07–4.28 (m, 5H), 5.10 (s, 2H), 5.2 (br s, 1H), 7.31–7.43 (m, 5H). TLC: R_f 0.07 (EtOAc:hexane, 2:1).

2.7.3. (2R)-*N*-Benzyloxycarbonyl-2-amino-3-hydroxypropyl acetate ((2R)-**2**)

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H), 2.39 (br s, 1H), 3.69 (m, 2H), 3.98 (m, 1H), 4.23 (d, 2H), 5.13 (s, 2H), 5.24 (br s, 1H), 7.32–7.43 (m, 5H). TLC: R_f 0.4 (EtOAc:hexane, 2:1), ee 98% (GLC), 60% yield $[\alpha]^{20}_D$ = −7.4 (EtOAc, c 0.797), $[\alpha]^{20}_D$ = +1.84 ($CHCl_3$, c 1.0).

2.7.4. (2S)-*N*-Benzyloxycarbonyl-2-amino-3-hydroxypropyl acetate ((2S)-**2**)

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H), 2.39 (br s, 1H), 3.69 (m, 2H), 3.98 (m, 1H), 4.23 (d, 2H), 5.13 (s, 2H), 5.24 (br s, 1H), 7.32–7.43 (m, 5H). TLC: R_f 0.4 (EtOAc:hexane, 2:1), ee 91% (GLC), 62% yield, $[\alpha]^{20}_D$ = −1.82 ($CHCl_3$, c 1.0).

3. Results and discussion

Acetylation of prochiral carboxybenzyl-2-amino-1,3-propanediol (cbz-serinol, **1**) with vinyl acetate as acyl donor catalyzed by different preparations of Pig pancreatic lipase in organic solvent was monitored by chiral HPLC/GLC as the reaction progressed (Scheme 1 and Fig. 1). Hydrolysis of diacetate **3** in phosphate buffer with acetonitrile as co-solvent and crude PPL gave the (*2S*)-monoacetate in 91% ee after 24 h (entry H in Table 1, reaction not shown in Scheme 1).

Composition of the reaction mixture during the first 3 h of acetylation of **1** is shown in Fig. 2 (Table 1, entry A). Both monoacetate enantiomers were formed, but virtually no diacetate. However, a small amount of diacetate (**3**) was observed after prolonged reaction time (3 days) (entry B).

The enantiomeric excess plot in Fig. 3 (entry A) shows that the (*2S*)-monoacetate (*2S*)-**2** is in high excess in the early stage of the reaction. At 85 min of conversion, the enantiomeric excess drops to zero. During further reaction the ee increases again, but now the

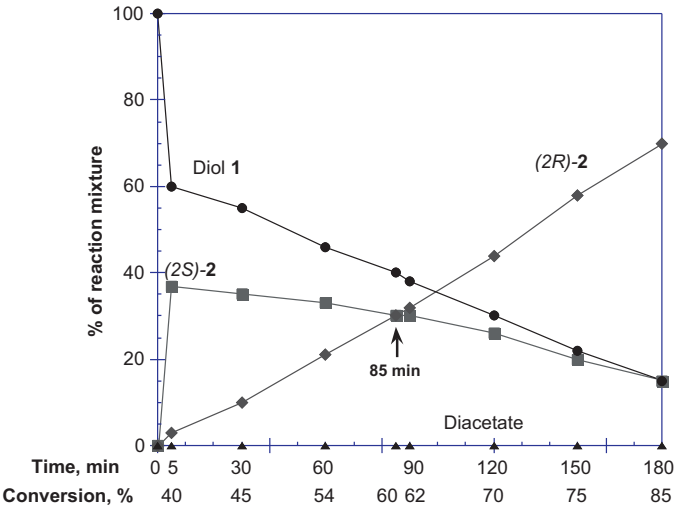

Fig. 2. Composition of reaction mixture in % of each compound, during acetylation (180 min) of **1** (circles) catalyzed by crude PPL in THF with vinyl acetate (equimolar amounts) (entry A, Table 1). (*2S*)-**2** (squares), (*2R*)-**2** (diamonds), diacetate (triangles).

(*2R*)-monoacetate, (*2R*)-**2**, is predominant (70% ee). Since no diacetate was observed during the first 3 h of reaction, the changing ee is not due to interconversion of (*2S*)-monoacetate via diacetate **3** to the (*2R*)-monoacetate. Acyl migration was shown to be negligible, which is anticipated due to the apolar solvent. Fadel and Arzel have reported that monoesters of serinol undergo racemization in acidic environments but not in apolar solvents [20]. Similar results have been obtained in our group [21].

When the acetylation of **1** with crude PPL was carried out in pure vinyl acetate (entry C, as previously reported by Choi and Borch [17]), only the (*2R*)-monoacetate, (*2R*)-**2**, was produced (in 98% ee) after 1.5 h. When the desymmetrization was catalyzed by purified PPL (Sigma Type VI-S, L0382, 20,000 U/mg) only the (*2S*)-monoacetate, (*2S*)-**2**, was formed (100% ee, entry E). This indicates that the (*R*)-selective enzyme is present in the crude PPL preparation. That the changing ee in reactions of the crude enzyme preparation is due to action of two different enzymes is supported by the fact that the (*2R*)-monoacetate was formed in excess ((*2R*)-**2**, 98% ee) when vinyl acetate was used as solvent. In this case it seems

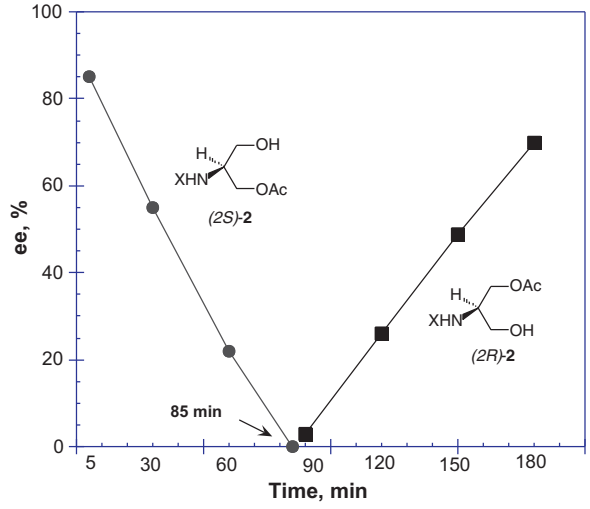

Fig. 3. Enantiomeric excess (ee) of monoacetates (*2S*)-**2** (circles) and (*2R*)-**2** (squares), entry A. At 85 min of reaction ee is zero. (*2S*)-**2** in excess before 85 min, (*2R*)-**2** in excess at 180 min (70% ee).

that vinyl acetate acts as an inhibitor of the (*S*)-selective enzyme. Both (*2S*)-**2** and (*2R*)-**2** were produced when vinyl acetate was used in equimolar amounts (entry A), or 1.2 times excess (entries D and E) and 4.3 times excess (entry B). Another batch of crude PPL (Lot 096K0721) was also used in desymmetrization reactions of **1** in THF both with vinyl acetate and vinyl butanoate, showing similar results as with crude PPL Lot 096K0747.

Characterization of compounds is in accordance with earlier reported data [17–19]. The absolute configuration of (*2R*)-**2** was determined by comparing optical rotation with previously reported values [17].

It is still interesting to reveal which of the enzymes in the crude preparation are responsible for the (*R*)-enantiomer. The PPL Type II enzyme preparation (Lot 096K0747, 358 U/mg) shows a slight chymotrypsin activity (7.2×10^{-2} U/mg) and general protease activity (2.5×10^{-4} U/mg). However, these enzymes are not responsible for the (*R*)-monoesters: Only the (*S*)-monobutanoate ((*2S*)-**4**, 100% ee, not shown in scheme) was produced in the reaction of **1** in THF with trypsin/chymotrypsin from pig pancreas as the catalyst and vinyl butanoate as the acyl donor (entry F). Pig liver esterase also produced solely the (*S*)-monobutanoate ((*2S*)-**4**, 100% ee, entry G) under the same conditions. As mentioned, Segura et al. reported that in kinetic resolution of ethyl 2-hydroxy-4-phenylbutanoate all three fractionated lipases (25-kDa, 33-kDa and PPL (52-kDa)) favored production of the (*S*)-monoester, however with low *E*-values [9]. Since the enantioselectivity of PPL towards this substrate is low, both enantiomers are actually produced. The fact that the enantiomeric excess calculations are based on the ee only at one single point of conversion, which is common, may also play a role. As mentioned, we have earlier observed that a change in enantioselectivity in kinetic resolutions was due to influence on the enzyme catalyst by (*R*)-alcohols in the medium, a possible non-competitive (or allosteric inhibition) [3,4]. Chiral compounds in the reaction medium is one of the oldest ways of obtaining enantioselectivity. Forty years ago Grignard reactions gave 70% ee when the reaction was carried out in the presence of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose [22].

Opposite from resolution, in desymmetrizations of prochiral compounds there are no chiral molecules present in the reaction mixture at the start of the reaction, but as the reaction proceeds, the concentration of pure enantiomers increases. Inhibition of enzymes by the formed products may also occur. In our experiments the slow-down of the (*S*)-selective enzyme may be a result of non-competitive inhibition by vinyl acetate and/or the produced (*R*)-monoester.

Although enantioselectivity and enantiomeric excess per definition are constant, concentrations of substrate and product enantiomers change throughout a reaction possibly affecting both catalysts and reaction medium. Our results show that it is always very important to monitor the reaction progress.

4. Conclusion

Action of lipases with opposite stereoselectivity seems to be the reason for the changing enantioselectivity of crude PPL in acetylation of **1**. Since crude PPL is a mixture of enzymes for digestive purposes, it is reasonable that it is able to hydrolyze both enantiomers of fats. However, the most abundant lipase in pig pancreas is (*S*)-selective towards **1**. It seems that this enzyme is the only active enzyme in the hydrolysis reaction of diacetyl cbz-serinol (**3**), since the only product was (*2S*)-**2**. Experiments are under way in order to establish which one of the 25-kDa and the 33-kDa lipase is giving rise to the (*R*)-monoesters of **1**. The (*S*)-selective enzyme is the faster reacting enzyme. Vinyl acetate acts as an inhibitor of the (*S*)-selective enzyme when used in large excess as

solvent, giving rise only to the (*R*)-monoacetate. However, since the (*S*)-selective enzyme "slows down" as the (*2R*)-monoester is produced when vinyl acetate is used in only 1.0–4.3 times excess, the (*2R*)-monoester may also play a role in the inhibition of the (*S*)-selective enzyme. Trypsin and chymotrypsin (from pancreas) and esterase from pig liver catalyzed the conversion of the (*2S*)-monobutanoate ((*2S*)-**4**) in 100% ee. Our experiments demonstrate that the crude PPL preparation gives rise to both monoester enantiomers in high enantiopurity depending on reaction conditions. When crude enzyme preparations are used, monitoring of the reaction progress is of utmost importance to determine the reaction of several enzymes in order to obtain the wanted enantiomer.

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References

[1] K. Faber, Biotransformations in Organic Chemistry, 6th ed., Springer-Verlag, Berlin, 2011, p. 42.
[2] U.T. Bornscheuer, R.J. Kazlauskas, Hydrolases in Organic Synthesis. Regio- and Stereoselective Biotransformations, 2nd ed., WILEY-VCH, 2005.
[3] E.E. Jacobsen, E.W. van Hellemond, A.R. Moen, L.C.V. Prado, T. Anthonsen, Tetrahedron Lett. 44 (2003) 8453.
[4] E.E. Jacobsen, L.S. Andresen, T. Anthonsen, Tetrahedron: Asymmetry 16 (2005) 847.
[5] E.E. Jacobsen, B.H. Hoff, T. Anthonsen, Chirality 12 (2000) 654.
[6] W.E. Ladner, G.M. Whitesides, J. Am. Chem. Soc. 106 (1984) 7250.
[7] M. Pregnolato, M. Terreni, I.E. de Fuentes, A. Alcantara Leon, P. Sabuquillo, R. Fernandez-Lafuente, J.M. Guisán, J. Mol. Catal. B: Enzym. 11 (2001) 757.
[8] T. Vorderwülbecke, K. Kieslich, H. Erdmann, Enzyme Microb. Technol. 14 (1992) 631.
[9] R.L. Segura, J.M. Palomo, C. Mateo, A. Cortes, M. Terreni, R. Fernández-Lafuente, J.M. Guisan, Biotechnol. Progr. 20 (2004) 825.
[10] T.A. Savidge, Biotechnology of Industrial Antibiotics, Marcel Decker, New York, 1984.
[11] V. Kerscher, W. Kreiser, Tetrahedron Lett. 28 (1987) 531.
[12] D. Breitgoff, K. Laumen, M.P. Schneider, J. Chem. Soc. Chem. Commun. (20) (1986) 1523.
[13] J.B.A. Van Tol, J.A. Jongejan, J.A. Duine, Biocatal. Biotransform. 12 (1995) 99.
[14] H. Tani, H. Ohishi, K. Watanabe, J. Agric. Food Chem. 43 (1995) 2796.
[15] J.D. Vaghjiani, T.S. Lee, G.J. Lye, M.K. Turner, Biocatal. Biotransform. 18 (2000) 151.
[16] K. Takabe, Y. Iida, H. Hiyoshi, M. Ono, Y. Hirose, Y. Fukui, H. Yoda, N. Mase, Tetrahedron Lett. 11 (2000) 4825.
[17] J.Y. Choi, R.F. Borch, Org. Lett. 9 (2007) 215.
[18] T. Wharton, L. Wilson, J. Bioorg. Med. Chem. 10 (2002) 3545.
[19] X. Cong, F. Hu, K.-G. Liu, Q.-J. Liao, Z.-J. Yao, J. Org. Chem. 70 (2005) 4514.
[20] A. Fadel, P. Arzel, Tetrahedron: Asymmetry 8 (1997) 283–291.
[21] B.J. Sjursnes, L. Kvittingen, T. Anthonsen, J. Am. Oil Chem. Soc. 72 (1995) 533.
[22] T.D. Inch, G.J. Lewis, G.L. Sainsbury, D.J. Sellers, Tetrahedron Lett. 41 (1969) 3657.