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Solvent-dependent reactivity in porcine pancreatic lipase (PPL)-catalyzed hydrolysis

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ABSTRACT

The solvent-dependent enzyme reactivity of porcine pancreatic lipase (PPL)-catalyzed hydrolysis was investigated using *trans*-**3** and *cis*-(3-(benzyloxymethyl)oxiran-2-yl)methyl acetate **4** as substrates. The conversion efficiency and enantioselectivity of the hydrolysis of these compounds were measured in three different types of enzymatic media: neat organic solvents, organic–aqueous mixture solvent systems, and an aqueous buffer solution. Comparison of the catalytic hydrolysis that occurred in 12 different organic solvents with log P values in the range of −1.10 to 3.50 revealed that the reactivity and selectivity of PPL-catalyzed hydrolysis toward (+)- and (−)-isomers were strongly affected by the solvent system. In neat organic solvents and organic–aqueous biphasic systems, (+)-*cis* oxirane acetate (+)-**4** was selectively hydrolyzed by PPL over the (−)-*cis* isomer. In contrast, hydrolysis of the (+)-*trans* substrate (+)-**3** was preferred in monophasic organic–aqueous systems and buffer solutions. The addition of water to the organic solvent increased the overall hydrolysis rate. However, hydrolysis in the aqueous buffer solution was considerably retarded since the organic substrates were insoluble in water. Therefore, control of the solvent system can result in the successful kinetic resolution of oxirane esters and their corresponding alcohols.

1. Introduction

Organic solvents have been used as enzyme reaction media to achieve various enzymatic transformations in organic synthesis applications.[2–4] The substrate selectivities of enzymes in organic media, including enantio-, prochiral, regio-, and chemoselectivities, differ profoundly in different solvents.[2–6] Numerous studies have shown that the catalysis properties of enzymes are different in organic media than in water, forming the basis of product selectivity.[1,5,6] Among the enzymes used for organic synthesis applications, porcine pancreatic lipase (PPL) has been utilized to catalyze a wide range of substrate hydrolysis and transesterification steps in monophase and multiphase solvent systems with high enantioselectivity.[4,7] Medium engineering (the optimization of enzyme selectivity in organic solvents) of PPL-catalyzed hydrolysis has also been attempted, with substrate specificities such as chain length, regio-, and enantioselectivity investigated in various media.[3,7–9]

Lipase-catalyzed transesterification reactions can be efficiently carried out in organic solvents, making them useful in preparative organic chemistry to attain asymmetric access to enantiomerically pure compounds.[7,9] However, in addition to transesterifications, concurrent catalytic hydrolysis of ester compounds occurs substantially even in organic solvents, since water molecules remaining on the enzyme surface[10] take part in the hydrolysis reaction. This hydrolysis reaction prevents transesterification reactions from completing and impedes their use in kinetic resolutions. Unlike transesterification reactions, catalytic hydrolysis requires water as a reactant and generally proceeds to completion in aqueous media. However, most organic substrates are insoluble in aqueous solutions and therefore enzyme-catalyzed hydrolysis is commonly carried out in aqueous–organic mixed-solvent systems to increase the solubility of the substrates. The minimum amount of water required to maintain enzyme structure and flexibility in organic media is about 0.05–3%.[11,12] Since the amount of water in the enzymatic media plays an important role in enzyme structure and reactivity, the enantioselectivity of enzymatic hydrolysis is also expected to depend on the water content in the reaction media and on the characteristics of the organic solvent. Therefore, herein we explored the enantioselectivity of PPL-catalyzed hydrolysis as a function of the water content and the organic solvent. The catalytic hydrolysis of racemic esters containing *trans* and *cis* oxirane alcohol units *rac*-**1** and *rac*-**2** (Fig. 1) was carried out in three different types of media: neat organic solvents, aqueous–organic systems (1:1 volume ratio), and aqueous solutions. A range of organic

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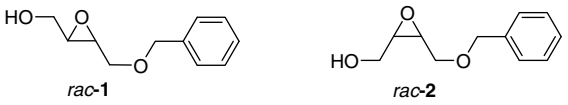


Figure 1.

solvents with log P values in the range of −1.10 to 3.50 was used. The conversion efficiency and enantioselectivity with respect to the substrate structures were investigated in these media.

trans and *cis* 3-(Benzyloxymethyl)oxirane-2yl methanol, **1** and **2**, are composed of fully functional four-carbon units with two stereogenic centers. These compounds are essential synthetic building blocks of biologically active natural products.[13] Enantiomerically pure compounds **1** and **2** are versatile intermediates for total synthesis via regio- and stereoselective ring opening, methathesis, or asymmetric aldol condensation.[14] For example, the aldehyde obtained by oxidation of **2** was used as the starting material in the total synthesis of cyclic guanidine sugars[15] and 3-methylated **2** acted as a key intermediate in the construction of the tricyclic core of phomactin A.[16] Compounds **1** and **2** were also used as precursors in the development of methodologies, such as the Baylis–Hillman reaction[17] and Mukaiyama aldolization.[18] The kinetic resolution of racemic oxirane compounds with biocatalysts can provide a simple, yet efficient synthetic method for oxirane enantiomers. However, only a few cases have been reported of the kinetic resolution of small molecular oxiranes using PPL.[19] Faigl et al. reported that the kinetic resolution of racemic *cis*-3-((benzyloxymethyl)oxiran-2-yl)methanol *rac*-**2** by PPL-catalyzed acetylation was achieved by the preferential reaction of a *cis*-(+)-oxirane methanol compound ((+)-**2**) in organic solvents.[20] Moseley and Staunton described the monohydrolysis of *meso*-oxiranedimethanol diesters by porcine-derived lipases, and also suggested a transformation to generate *cis*-(+)-epoxide methanol intermediates.[21] Here, the reactions that were monitored are shown in Scheme 1. In these reactions, **1** and **2** are produced by PPL-catalyzed hydrolysis from their acetate esters **3** and **4**, respectively. The reactivity and enantioselectivity of the reactions were investigated in various media.

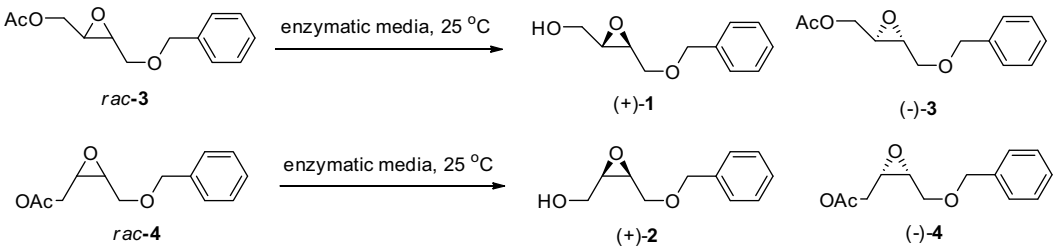
2. Results and discussion

2.1. Preparation of substrates for enzymatic hydrolysis

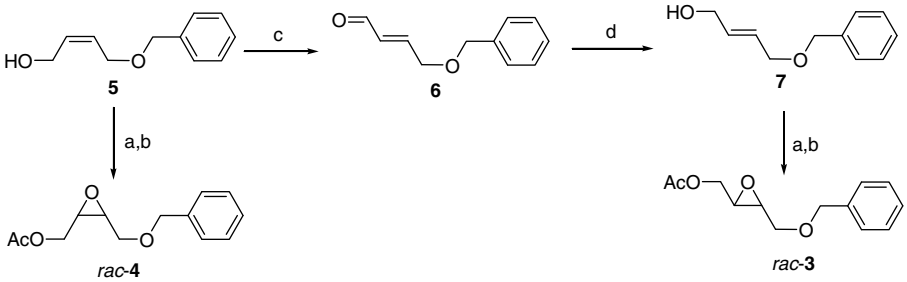
trans- and *cis*-Monobenzylated oxirane dimethanol esters *rac*-**3** and *rac*-**4** were prepared from monobenzylated *cis*-butene-1,4-diol, as shown in Scheme 2. Epoxidation of **5** and **7** was performed using *m*-CPBA followed by acetylation to produce the monobenzylated oxiranes *rac*-**4** and *rac*-**3**, respectively. For the *trans* ester *rac*-**3**, compound **5** was converted to *trans*-α,β-unsaturated olefin compound **6** due to conjugation during the Dess–Martin periodinane[22] oxidation. Next, compound **6** was reduced to the allylic alcohol **7** with a high yield.

2.2. Enzyme selectivity of regioisomers in different media

A major advantage of using organic media in enzyme-catalyzed reactions is improved substrate solubility. Substrates that are poorly soluble in water can have a greater chance of interacting with the enzyme in organic solvents at the expense of enzyme reactivity, which often accompanies the structural changes that occur in organic solvents. Partial replacement of the organic solvent with aqueous media might be preferable for the structural stability of the enzyme, while keeping the hydrophobic substrates dissolved in the solution. Denaturation of the enzyme can be avoided in this mixed system and the catalytic activity of the enzyme can be enhanced, particularly for hydrolases, because water provides another reactant for the hydrolysis.[1] Here, two different kinds of solvent environments should be considered: polar organic solvents miscible with water, and non-polar organic solvents that are partially miscible or immiscible when mixed with water, resulting in a biphasic system. For PPL-catalyzed hydrolysis, in a homogeneous solvent system composed of water and a water-miscible organic solvent, the probability of substrates encountering each other is improved, since both the enzyme and substrates are dissolved in the solution. However, the reactivity and specificity of the enzyme cannot be easily predicted. The active-site conformation and structural rigidity of the enzyme are altered in these mixed solvents and even small structural changes can have



Scheme 1. Enzymatic hydrolysis reactions investigated in this study.



Scheme 2. Synthesis of substrates **3** and **4**. Reagents and conditions: (a) *m*CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C to rt, 18 h, 78%; (b) acetic anhydride, acetic acid, rt, 12 h, 95%; (c) Dess–Martin periodinane, CH_2Cl_2, rt, 20 h, 84%; (d) $NaBH_4$, MeOH, 0 °C to rt, 20 min, 98%.

large effects on the reactivity and specificity. In contrast, in a water–organic biphasic solvent system, PPL can adopt the native structure that it has in aqueous media.[5] However, the opportunities for interactions with substrates are significantly reduced, since the reaction only takes place at the interface.[23] In aqueous media, the encounters of PPL with substrates are expected to be even less frequent due to the decreased interfacial area.

2.2.1. PPL-catalyzed hydrolysis in neat organic solvents

In general, the catalytic activities of enzymes in neat organic solvents are thought to be far lower than in water.[2] In anhydrous organic solvents, lyophilized or freeze-dried enzymes lose their lubricating agent, water, which covers the enzyme surface. The enzyme tends to denature, resulting in a very rigid conformation.[1] This low flexibility of the enzyme structure diminishes the enzyme activity in neat organic solvents, even though crystalline enzymes essentially retain their native structures.[4]

The results of PPL-catalyzed hydrolysis in various organic media are summarized in Table 1 and the enantiomeric ratios obtained using *rac*-**3** and *rac*-**4** as substrates and two reaction times are compared in Figure 2. Note that in nearly anhydrous organic solvents, a substantial amount of enantiomeric catalytic activity of PPL was observed for the hydrolysis of *rac*-**4**, whereas that for *rac*-**3** was negligible. Both oxirane esters, **3** and **4**, were hydrolyzed slowly and inefficiently, probably due to the limited amount of reactant water available in the anhydrous media. With *rac*-**3**, as

Table 1
PPL-catalyzed hydrolysis in pure organic solvents[a]

Entry	Substrate	Solvent[b]	log *P*[c]	3 h			48 h			Product
				Conversion[d] (%)	ee_p[e] (%)	*E*[f]	Conversion[d] (%)	ee_p[e] (%)	*E*[f]	
1	**3**	1,4-Dioxane	−1.10	14.8	34.0	2.04	59.7	3.8	1.17	(+)-**1**
2	**3**	Methanol	−0.76	6.9	50.8	3.08	7.7	44.2	2.60	(+)-**1**
3	**3**	Acetonitrile	−0.33	10.5	22.6	1.70	39.5	3.4	1.10	(+)-**1**
4	**3**	Ethanol	−0.24	47.9	54.6	4.30	78.8	0.6	1.14	(−)-**1**
5	**3**	Acetone	−0.23	12.2	35.1	2.06	69.2	1.5	1.07	(−)-**1**
6	**3**	Tetrahydrofuran	0.49	21.6	44.5	2.69	62.3	3.6	1.16	(+)-**1**
7	**3**	Dichloromethane	0.60	17.7	23.0	1.60	58.2	15.3	1.44	(+)-**1**
8	**3**	Ethyl ether	0.85	28.1	12.9	1.31	68.1	3.2	1.07	(−)-**1**
9	**3**	Toluene	2.50	20.1	38.8	2.33	33.2	4.5	1.11	(+)-**1**
10	**3**	Xylene	3.10	19.7	28.0	1.83	40.6	2.9	1.10	(+)-**1**
11	**3**	Cyclohexane	3.20	18.2	12.0	1.29	27.2	2.3	1.07	(−)-**1**
12	**3**	Hexane	3.50	29.0	23.9	1.70	26.0	1.8	1.05	(−)-**1**
13	**4**	1,4-Dioxane	−1.10	27.6	12.1	1.33	72.0	20.1	1.77	(+)-**2**
14	**4**	Methanol	−0.76	19.4	16.3	1.45	24.8	25.7	1.67	(+)-**2**
15	**4**	Acetonitrile	−0.33	16.6	10.9	1.31	80.0.	27.5	2.42	(+)-**2**
16	**4**	Ethanol	−0.24	56.7	35.2	2.40	95.4	0.3	1.29	(−)-**2**
17	**4**	Acetone	−0.23	22.0	69.3	5.73	42.8	82.7	12.67	(+)-**2**
18	**4**	Tetrahydrofuran	0.49	22.1	61.1	4.26	47.5	83.4	13.77	(+)-**2**
19	**4**	Dichloromethane	0.60	28.9	63.7	4.83	84.3	79.3	17.24	(+)-**2**
20	**4**	Ethyl ether	0.85	33.1	9.2	1.26	76.2	0.7	1.18	(+)-**2**
21	**4**	Toluene	2.50	23.7	37.8	2.23	59.7	39.8	2.84	(+)-**2**
22	**4**	Xylene	3.10	37.7	26.7	1.78	59.5	95.9	67.50	(+)-**2**
23	**4**	Cyclohexane	3.20	23.5	76.6	7.95	67.2	83.3	16.66	(+)-**2**
24	**4**	Hexane	3.50	23.0	73.3	6.80	42.4	76.0	8.69	(+)-**2**

[a] The hydrolysis procedure is described in Section 4.
[b] Organic solvents were prepared according to the general procedure described in Section 4.
[c] Ref. 1.
[d] Conversion rates were determined based on HPLC analysis using a chiral column.
[e] Enantiomeric excess values were determined based on HPLC analysis using a chiral column.
[f] $E = \dfrac{\ln\left[\frac{1-ee_s}{1+(ee_s/ee_p)}\right]}{\ln\left[\frac{1-ee_s}{1+(ee_s/ee_p)}\right]}$.

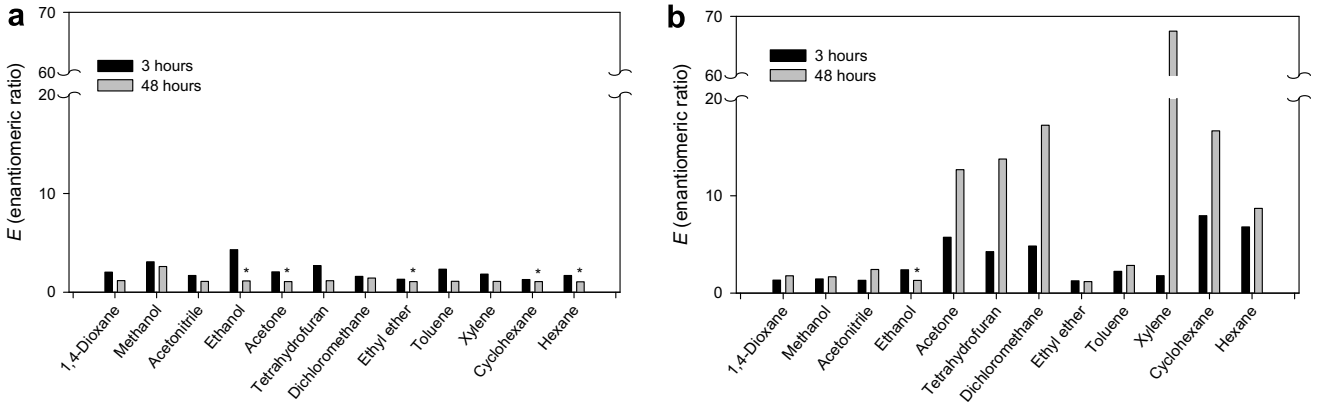


Figure 2. Trends in *E* (enantiomeric ratio) values in the hydrolysis of *rac*-**3** (a) and *rac*-**4** (b) by PPL in organic solvents. Asterisks indicate that the opposite enantiomer is in excess.

the reaction proceeded, the percentage of conversion to (+)-**1** increased, but the enantiomeric excess (ee) value for (+)-**1** decreased, resulting in small *E* values (Fig. 2a). This racemization of products indicates that the conversion rate to (+)-**1** is higher than that to (−)-**1**, but the differences in their rates were not great enough to result in kinetic resolution. With *rac*-**4**, enantioselectivity was higher than for *rac*-**3** in most non-polar organic solvents. The general conversion rate to (+)-**2** was much higher than that to (−)-**2**, resulting in high ee% values. However, the absolute stereochemistry was not confirmed experimentally, even though (−)-**1** was obtained. The stereochemistry of (+)-**2** has been reported.[20] The conversion percentage in the 12 organic solvents ranged from 3 to 48 h, with little change in the ee% (enantiomeric excess) values. Particularly in xylene, *rac*-**4** was enantioselectively converted to (+)-**2** by PPL-catalyzed hydrolysis with a high *E* value, 67.5, at 48 h. This high selectivity demonstrates that PPL maintains enantioselective reactivity even in neat organic solvents.

2.2.2. PPL-catalyzed hydrolysis in aqueous buffer and organic solvents

The reaction solvent gradually acidifies as the monobenzylated epoxy esters **3** and **4** are hydrolyzed to the corresponding alcohols and acids. Giner et al. described in a biomimetic study that *trans* epoxy alcohols can be converted to 2-methyl-D-erythritol via [2,2,1]-bicyclic orthoester intermediate rearrangements under acidic conditions.[24] Accordingly, we designed a solvent system in

Table 2
PPL-catalyzed hydrolysis in a mixture of buffer (pH 7.2, phosphate) and organic solvent (1:1, v/v)[a]

Entry	Substrate	Solvent[b]	log *P*[c]	3 h			48 h			Product
				Conversion[d] (%)	ee_p[e] (%)	*E*[f]	Conversion[d] (%)	ee_p[e] (%)	*E*[f]	
1	**3**	1,4-Dioxane	−1.10	88.6	44.7	3.07	84.1	15.9	1.73	(+)-**1**
2	**3**	Methanol	−0.76	84.4	68.7	6.41	60.7	66.6	4.82	(−)-**1**
3	**3**	Acetonitrile	−0.33	94.8	60.4	11.26	72.4	84.1	15.84	(−)-**1**
4	**3**	Ethanol	−0.24	89.9	40.2	4.85	46.4	41.5	4.40	(−)-**1**
5	**3**	Acetone	−0.23	82.2	16.8	1.52	82.8	13.2	1.50	(+)-**1**
6	**3**	Tetrahydrofuran	0.49	28.2	64.5	2.62	31.3	67.6	7.99	(+)-**1**
7	**3**	Dichloromethane	0.60	86.6	19.9	1.82	96.5	15.9	1.55	(+)-**1**
8	**3**	Ethyl ether	0.85	88.8	17.0	1.56	98.2	14.9	2.38	(+)-**1**
9	**3**	Toluene	2.50	81.1	89.6	32.55	94.0	7.8	1.57	(−)-**1**
10	**3**	Xylene	3.10	61.4	12.9	1.53	89.4	67.3	12.22	(+)-**1**
11	**3**	Cyclohexane	3.20	88.7	73.9	15.40	99.3	0.8	1.08	(+)-**1**
12	**3**	Hexane	3.50	67.2	1.1	1.19	99.4	0.6	1.07	(+)-**1**
13	**4**	1,4-Dioxane	−1.10	16.4	23.7	1.75	20.9	47.2	3.12	(+)-**2**
14	**4**	Methanol	−0.76	32.6	58.5	4.46	52.1	79.4	12.14	(+)-**2**
15	**4**	Acetonitrile	−0.33	11.2	18.5	1.40	8.5	2.7	1.10	(−)-**2**
16	**4**	Ethanol	−0.24	24.9	57.5	4.15	26.8	69.7	6.53	(+)-**2**
17	**4**	Acetone	−0.23	33.7	63.5	5.37	50.5	83.6	15.20	(+)-**2**
18	**4**	Tetrahydrofuran	0.49	11.9	22.7	1.62	8.9	18.3	1.49	(−)-**2**
19	**4**	Dichloromethane	0.60	28.0	39.5	2.57	45.9	76.3	10.22	(+)-**2**
20	**4**	Ethyl ether	0.85	76.7	97.0	99.73	91.3	82.2	26.55	(+)-**2**
21	**4**	Toluene	2.50	89.9	93.4	58.32	69.6	81.9	17.86	(+)-**2**
22	**4**	Xylene	3.10	89.8	95.3	48.64	82.6	98.0	153.91	(+)-**2**
23	**4**	Cyclohexane	3.20	91.9	96.1	103.27	90.2	99.5	>200	(+)-**2**
24	**4**	Hexane	3.50	88.7	89.0	34.67	89.8	96.2	129.95	(+)-**2**

[a] The hydrolysis procedure is described in Section 4.
[b] Organic solvents were distilled according to the general procedure described in Section 4 prior to mixing with buffer.
[c] Ref. 1.
[d] Conversion rates were determined based on HPLC analysis using a chiral column.
[e] Enantiomeric excesses values were determined based on HPLC analysis using a chiral column.
[f] $E = \dfrac{\ln\left[\frac{1-ee_s}{1+(ee_s/ee_p)}\right]}{\ln\left[\frac{1-ee_s}{1+(ee_s/ee_p)}\right]}$.

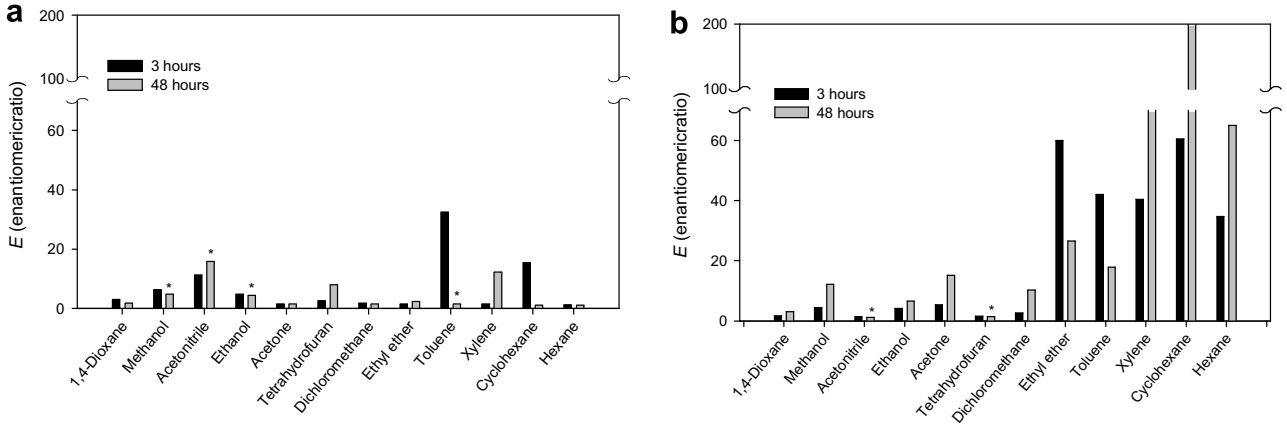


Figure 3. Trends in *E* (enantiomeric ratio) values in the hydrolysis of *rac*-**3** (a) and *rac*-**4** (b) by PPL in phosphate buffer (pH 7.2) and a 1:1 (v/v) organic solvent mixture. Asterisks indicate that the opposite enantiomer is in excess.

which the acid product could be neutralized immediately to avoid further cleavage of the oxirane ring in the substrates. Here, a phosphate buffer (pH 7.2) was introduced and mixed with different organic solvents in a 1:1 volume ratio for optimization of the PPL-catalyzed hydrolysis reactions at 25 °C.

Figure 3 shows the improved kinetic resolution that occurred in aqueous buffer–organic solvents. As summarized in Table 2, both *rac*-**3** and *rac*-**4** showed more efficient conversions than in neat organic solvents. The conversion efficiency of *rac*-**3** in the aqueous–organic solutions was higher, with larger ee% values than in organic solvents. In homogeneous mixed-solvent systems, such as 1,4-dioxane, methanol, acetonitrile, ethanol, and acetone, the conversion efficiencies of *rac*-**3** were even better than for *rac*-**4** in the same solvent systems. Nevertheless, the enantioselectivity for *rac*-**3** was not high enough for an efficient kinetic resolution in these solvent systems (Fig. 3a). In biphasic solvent systems (aqueous buffer mixed with dichloromethane, ethyl ether, toluene, xylene, cyclohexane, or hexane), the conversion of *rac*-**3** was also highly effective at 3 and 48 h, but the ee% was much lower at 48 h, except in the xylene biphasic system. In the xylene buffer system, the conversion percentage and ee% increased steadily through 48 h.

In contrast, the reactivity of PPL toward *rac*-**4** was significantly different from that of *rac*-**3**. In mixed-solvent systems containing relatively polar organic solvents, whose log P values were lower than 1.0, the conversion of *rac*-**4** to (+)-**2** was less efficient than the conversion of *rac*-**3** to (+)-**1**. However, in solvent systems containing the non-polar organic solvents ethyl ether and hexane, the conversion efficiency and ee% of *rac*-**4** were both large, resulting in large *E* values (Fig. 3b). Among these non-polar solvents, the solvent system containing cyclohexane exhibited the largest *E* value, >200, at 48 h. Hence, the results indicate that PPL enantioselectively catalyzes the hydrolysis of *rac*-**4** in an organic buffer mixed-solvent system with a highly non-polar organic solvent. These solvent mixtures are ideal environments for the kinetic resolution of *rac*-**4**.

2.2.3. PPL-catalyzed hydrolysis in buffer aqueous solutions

The enantioselective hydrolysis of the racemic oxirane compounds *rac*-**3** and *rac*-**4** by PPL was performed in the phosphate buffer (pH 7.2) at 25 °C (Fig. 4). In aqueous media, hydrophobic substrates drift as microscopic granules until they encounter enzymes. Therefore, the hydrolysis of organic compounds in water generally proceeds very slowly. As anticipated, the observed reaction rates were much slower than in solvent systems containing organic solvents. We discovered that longer reaction times, for example, 120 h, can produce an efficient kinetic resolution with *rac*-**3**, as shown in Fig. 4. The steep slope of *rac*-**4** indicates that PPL slowly, enantioselectively hydrolyzes the *trans*-isomers in neat buffer solutions. Under these circumstances, the enzyme selectivity for the *cis*-compound *rac*-**4** was lower than that for the *trans*-compound, although the conversion rate slowly increased from 3 h to 120 h. The enantioselective excess values and the conversion rates of *rac*-**3** hydrolysis improved steadily until the *E* value reached 87.6 at 120 h. Therefore, we conclude that in aqueous media,*trans*-compounds are better substrates for PPL, and the enzyme can distinguish enantiomers of the *trans* compounds better than those of *cis* compounds.

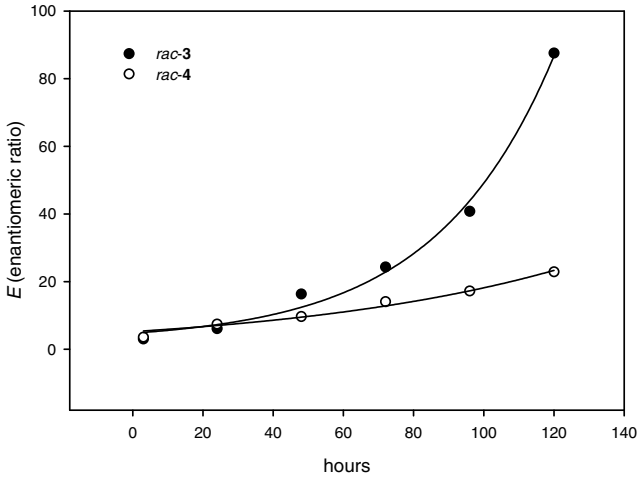


Figure 4. PPL-catalyzed hydrolysis of *rac*-**3** and *rac*-**4** in buffer solution (pH 7.2).

3. Conclusions

We have found that PPL has a discriminating hydrolysis reactivity toward substrates depending on the enzymatic medium, even when the substrates have very similar molecular skeletons. In neat organic solvent and organic–aqueous systems, the reactivity of PPL is more suited for the hydrolysis of the *rac*-**4** (*cis*) compound than the *rac*-**3** (*trans*) compound. The kinetic resolution of *rac*-**4** and its corresponding alcohol (+)-**2** was obtained efficiently in an organic buffer solvent system, in particular cyclohexane, hexane, and xylene biphasic systems. In contrast, in aqueous buffer media (pH 7.2), the reactivity of PPL toward *rac*-**3** was ideal for the kinetic resolution of alcohol (+)-**1**. In view of these results, we conclude that in the presence of organic solvents, PPL adopts a structure that is more suitable for the hydrolysis of *rac*-**4**, but in aqueous buffer media, the enzyme retains its native structure, which is better for the hydrolysis of *rac*-**3**. Hence, the extent of the aqueous nature of the medium can be an important factor in choosing hydrolysis enzymatic media for kinetic resolution. By manipulating the water content in the enzymatic media, a more efficient kinetic resolution can be achieved.

4. Experimental

4.1. General

Chemical reagents and PPL were obtained from Aldrich (Milwaukee, WI, USA) and Sigma (St. Louis, MO, USA), respectively. Tetrahydrofuran and ethyl ether were distilled over sodium metal and benzoquinone under argon; dichloromethane, acetonitrile, and toluene were distilled over calcium hydride. Other solvents were purchased from Aldrich, J. T. Baker (Phillipsburg, NJ, USA), and Fisher (Hampton, NH, USA) as absolute grades. Enzymatic reactions were performed in a Jeio Tech (Seoul, Korea) SI-300R incubatory orbital shaker at 160 rpm and 25 °C. Conversion rates and enantiomeric excess values were determined by chiral HPLC analysis (1200 LC; Hewlett–Packard, Palo Alto, CA, USA), using a Chiracel OD column (250 × 4.6 mm i.d.; Daicel Chemical Industries Ltd., Osaka, Japan) and a general mobile phase of a mixture of hexane and isopropanol (86:14) with a 1 ml/min flow rate. Optical rotations were measured using a digital polarimeter (DIP-1000; Jasco, Tokyo, Japan). NMR spectra were recorded on a spectrometer (Avance 400; Bruker, Bremen, Germany; NMR 500; Varian, Palo Alto, CA, USA), using $CDCl_3$ as the solvent. Chemical shifts are given in ppm relative to the tetramethylsilane signal. Coupling constants are given in Hertz. IR spectra were taken on a Jasco 420 FT-IR spectrometer. Thin-layer chromatography was performed using pre-coated silica gel plates (Merck 60 F_{254}; Merck, Darmstadt, Germany). Column chromatography was performed on Silica Gel 60 (0.040–0.063 mm, 230–400 mesh; Merck), employing hexane and ethyl acetate as developing solvents.

4.2. Preparation of (*cis*-3-(benzyloxymethyl)oxirane-2-yl)methyl acetate *rac*-4

A CH_2Cl_2 (40 ml) solution of **5** (2.425 g, 13.61 mmol) was added to $NaHCO_3$ (2172 mg, 25.85 mmol) at 0 °C in one portion and stirred for 5 min. Then, *m*CPBA (2818 mg, 16.33 mmol) was added to the suspension and stirred for 3 h, during which time the temperature rose to room temperature. The solution was quenched with saturated aqueous $NaHCO_3$ (25 ml) and diluted with CH_2Cl_2. The organic layer was separated and the aqueous layer extracted three times with CH_2Cl_2. The combined organic layer was dried with brine and anhydrous $MgSO_4$. The solvent was evaporated in vacuo, and the residue was separated by column chromatography (3:1 *n*-hexane/EtOAc) to afford the oxirane compound as a colorless oil, with a 78% yield. The CH_2Cl_2 solution (40 ml) of the oxirane compound (2062 mg, 10.61 mmol) was pyridine (1.28 ml, 15.94 mmol), DMAP (259.64 mg, 2.12 mmol), and $(CH_3CO)_2O$ (1.30 ml, 12.74 mmol) at 0 °C, and it was stirred for 6 h at room temperature. Then, the mixture was diluted with dichloromethane (40 ml) and washed with water. The organic layer was combined, the solvent was evaporated in vacuo, and the residue was separated by column chromatography (4:1 *n*-hexane/EtOAc) to afford 1.318 g acetylated racemic epoxide **4** in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31 (m, 5H, –C_6H_5), 4.03 (q, 2H, –O–CH_2–C_6H_5, $^4J_{am}$ = 12.3 Hz, $^2J_{aa}$ = 33.1 Hz), 4.32 (q, 1H, –H(O)C–CH_2–O–, $^4J_{am}$ = 12.3 Hz, $^3J_{ab\text{-}trans}$ = 3.8 Hz), 4.03 (q, 1H, –H(O)C–CH_2–O–, $^4J_{am}$ = 12.3 Hz, $^3J_{ab\text{-}cis}$ = 7.0 Hz), 3.71 (q, 1H, –O–CH_2–C(O)H–, $^3J_{ab\text{-}trans}$ = 4.1 Hz), 3.59 (q, 1H, –O–CH_2–C(O)H–, $^3J_{ab\text{-}cis}$ = 6.1 Hz), 3.27 (m, 2H, –C(O)*H*–C(O)*H*–, $^3J_{ab\text{-epoxide}}$ = 5.1 Hz, $^3J_{ab\text{-}cis}$ = 6.1 Hz, $^3J_{ab\text{-}trans}$ = 4.1 Hz, $^3J_{ab\text{-}cis}$ = 7.0 Hz, $^3J_{ab\text{-}trans}$ = 3.8 Hz), 2.09 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.69, 137.60, 129.82, 128.49, 127.90, 127.81, 126.85, 71.52, 67.83, 62.60, 54.73, 53.02, and 20.73.

4.3. Preparation of *trans*-4-(benzyloxy)but-2-enal 6

A 75-ml CH_2Cl_2 solution of **5** (1.964 g, 11.02 mmol) was added to Dess–Martin periodinane[22] (5.610 g, 13.22 mmol) and stirred at room temperature for 20 h. After filtering the reaction mixture, the solvent was evaporated and the residue separated immediately by column chromatography with a mixture of *n*-hexane and ethyl acetate (10:1) as a developing solvent. Then, 1.63 g of a colorless liquid product was obtained with 84% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.56 (d, 1H, O=C*H*, $^3J_{ab}$ = 7.8 Hz), 7.34 (m, 5H, –C_6H_5), 6.86 (m, 1H, –CH=C*H*–O–, $^3J_{ab\text{-}trans}$ = 15.8 Hz, $^3J_{ab}$ = 7.8 Hz, $^4J_{am}$ = 4.0 Hz), 6.41 (m, 1H, O=C(H)–C*H*=CH–, $^3J_{ab\text{-}trans}$ = 15.8 Hz, $^3J_{ab}$ = 4.0 Hz), 4.60 (s, 2H, –O–CH_2–C_6H_5), 4.30 (q, 2H, =CH–CH_2–O–, $^3J_{ab}$ = 4.0 Hz, $^4J_{am}$ = 4.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 193.64, 147.09, 137.36, 131.72, 129.00, 128.53, 127.99, 127.90, 127.70, 72.88, and 68.52.

4.4. Preparation of *trans*-4-benzyloxy-but-2-en-1-ol 7

Aldehyde compound **6** (624 mg, 3.55 mmol) was dissolved in methanol (12 ml), and $NaBH_4$ (268.5 mg, 7.1 mmol) was added to the solution at 0 °C. Then, the solution was stirred for 20 min at room temperature. The solvents were evaporated and the residue was diluted with ether. The crude solution was extracted with $NaHCO_3$ (5 ml), distilled water (5 ml), and brine (5 ml). The organic extracts were dried with $MgSO_4$, evaporated in vacuo, and chromatographed (3:1 *n*-hexane/EtOAc), with an 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 (m, 5H, –C_6H_5), 7.26 (s, 1H, –O*H*), 5.81 (m, 1H, –CH_2–C*H*=CH–CH_2–, $^3J_{ab\text{-}trans}$ = 17.8 Hz, $^3J_{ab}$ = 6.0 Hz), 5.76 (m, 1H, –CH_2–CH=C*H*–CH_2–, $^3J_{ab\text{-}trans}$ = 17.8 Hz, $^3J_{ab}$ = 6.2 Hz), 4.53 (s, 2H, –O–CH_2–C_6H_5), 4.18 (d, 2H, HO–CH_2–CH=, $^3J_{ab}$ = 6.2 Hz), 4.10 (d, 2H, =CH–CH_2–O–, $^3J_{ab}$ = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.86, 132.37, 128.49, 128.36, 127.88, 72.53, 65.68, and 58.80.

4.5. Preparation of (*trans*-3-(benzyloxymethyl) oxiran-2-yl)-methyl acetate *rac*-3

A CH_2Cl_2 (40 ml) solution of **7** (2.425 g, 13.61 mmol) was added to $NaHCO_3$ (2172 mg, 25.85 mmol) at 0 °C in one portion and stirred for 5 min. Then, *m*CPBA (2.818 g, 16.33 mmol) was added to the suspension and stirred for 3 h, during which time the temperature rose to room temperature. The solution was quenched with saturated aqueous $NaHCO_3$ (25 ml) and diluted with CH_2Cl_2. The organic layer was separated and the aqueous layer extracted three times with CH_2Cl_2. The combined organic layer was dried with brine and anhydrous $MgSO_4$, the solvent was evaporated in vacuo, and the residue was separated by column chromatography (3:1 *n*-hexane/EtOAc) to afford 2.062 g of the oxirane compound with 78% yield as a colorless oil. The CH_2Cl_2 solution (40 ml) of the oxirane compound (2.062 g, 10.61 mmol) was pyridine (1.28 ml, 15.94 mmol), DMAP (259.64 mg, 2.12 mmol), and $(CH_3CO)_2O$ (1.30 ml, 12.74 mmol) at 0 °C and was stirred for 6 h at room temperature. Then, the mixture was diluted with dichloromethane (40 ml) and washed with water. The organic layer was combined, the solvent evaporated in vacuo, and the residue was separated by column chromatography (4:1 *n*-hexane/EtOAc) to afford 1.318 g of acetylated racemic epoxide **3** with 99% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.35 (m, 5H, –C_6H_5), 4.59 (q, 2H, –O–CH_2–C_6H_5, $^4J_{am}$ = 12.3 Hz, $^2J_{aa}$ = 33.1 Hz), 4.33 (q, 1H, –H(O)C–CH_2–O–, $^4J_{am}$ = 12.3 Hz, $^3J_{ab\text{-}trans}$ = 3.83 Hz), 4.03 (q, 1H, –H(O)C–CH_2-O–, $^4J_{am}$ = 12.3 Hz, $^3J_{ab\text{-}cis}$ = 6.98 Hz), 3.72 (q, 1H, –O–CH_2–C(O)H–, $^3J_{ab\text{-}trans}$ = 4.14 Hz), 3.60 (q, 1H, –O–CH_2–C(O)H–, $^3J_{ab\text{-}cis}$ = 6.06 Hz), 3.28 (m, 2H, –C(O)*H*–C(O)*H*–, $^3J_{ab\text{-epoxide}}$ = 5.14 Hz, $^3J_{ab\text{-}cis}$ = 6.06 Hz, $^3J_{ab\text{-}trans}$ = 4.14 Hz, $^3J_{ab\text{-}cis}$ = 6.98 Hz, $^3J_{ab\text{-}trans}$ = 3.83 Hz), 2.10 (t, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.01, 128.75, 128.72, 128.16, 128.08, 128.03, 73.64, 68.08, 66.31, 54.96, 52.97, and 21.02.

4.6. General procedure for PPL-catalyzed hydrolysis

Racemic compound **3** or **4** (1.066 g, 8.49 mmol) was dissolved in solvent (25 ml) and fixed in an incubatory orbital shaker at 25 °C. PPL (1066 mg) was added to the solution and rotated at 160 rpm. The solution was filtered with a Celite-packed glass filter to remove the PPL, and the organic layer was separated. The organic layer was washed with 20 ml of distilled water and then dried with brine and anhydrous magnesium sulfate. Then, the solvent was evaporated in vacuo and separated by chromatography (3:1 *n*-hexane/EtOAc) to afford chiral epoxide **1** or **2**.

For **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (m, 5H, –C_6H_5), 4.55 (q, 2H, –O–CH_2–C_6H_5, $^4J_{am}$ = 11.9 Hz, $^2J_{aa}$ = 34.0 Hz), 3.64 (m, 4H, –H(O)C–CH_2–OH, –H(O)C–CH_2–O–, $^3J_{ab}$ = 5.4 Hz, $^3J_{ab}$ = 6.0 Hz), 3.26 (m, 1H, –C(O)*H*–C(O)H–, $^3J_{ab\text{-epoxide}}$ = 9.1 Hz, $^3J_{ab}$ = 5.4 Hz), 3.19 (m, 1H, –C(O)H–C(O)*H*–, $^3J_{ab\text{-epoxide}}$ = 9.1 Hz, $^3J_{ab}$ = 6.0 Hz), 2.83 (s, 1H, –O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.49, 131.64, 129.80, 128.53, 127.98, 127.89, 73.42, 68.06, 60.58, 55.90, 54.92. ee% 92.3%; $[\alpha]_D^{24} = +21.65$ (*c* 0.56, EtOAc), HPLC chiral column retention time, 25.2 min.

For **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35 (m, 5H, –C_6H_5), 4.55 (q, 2H, –O–CH_2–C_6H_5, $^4J_{am}$ = 11.8 Hz, $^2J_{aa}$ = 34.6 Hz), 3.64 (m, 4H, –H(O)C–CH_2–OH, –H(O)C–CH_2–O–, $^3J_{ab}$ = 5.0 Hz, $^3J_{ab}$ = 6.0 Hz), 3.29 (m, 1H, –C(O)*H*–C(O)H–, $^3J_{ab\text{-epoxide}}$ = 17.0 Hz, $^3J_{ab}$ = 5.0 Hz), 3.23 (m, 1H, –C(O)H–C(O)*H*–, $^3J_{ab\text{-epoxide}}$ = 17.0 Hz, $^3J_{ab}$ = 6.0 Hz), 2.11 (s, 1H, –O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 137.37, 128.58, 128.05, 127.91, 73.52, 68.09, 60.76, 54.74, and 54.26. ee% 99.5%; $[\alpha]_D^{24} = +20.5$ (*c* 0.45, EtOAc), HPLC chiral column retention time, 18.7 min.

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