Chemical Papers
https://doi.org/10.1007/s11696-019-00796-9

ORIGINAL PAPER

Lipase-catalyzed hydrolysis of 2-(4-hydroxyphenyl)propionic acid ethyl ester to (*R*)-(−)-2-(4-hydroxyphenyl)propanoic acid

Xin Yuan[1] · Panliang Zhang[1] · Guangyong Liu[1] · Weifeng Xu[1] · Kewen Tang[1]

Received: 9 January 2019 / Accepted: 22 April 2019

Abstract
Stereoselective hydrolysis of (±)-2-(4-hydroxyphenyl)propionic acid ethyl ester (2-HPPAEE) by lipase catalyzed in aqueous system was investigated. Lipase AK with higher catalytic activity and enantioselectivity was selected as catalyst. Simultaneously, factors affecting the conversion of substrate (*c*) and the enantiomeric excess of product (ee_p) were optimized. The optimal conditions were established, involving 45 °C of temperature, 5.5 of pH, 10 mg of lipase AK dosage, 0.04 mmol of substrate dosage and 40 h of reaction time. Under the optimum conditions, *c* and ee_p could reach up to 49% and 98%, respectively.

Keywords Kinetic resolution · Lipase AK · Hydrolysis · 2-(4-Hydroxyphenyl) propionic acid ethyl ester

Introduction

Chiral drugs have taken up about one-third of the global drug market. The market of optically pure drugs is increasing dramatically, so the development of chiral separation technology is crucial. Generally, the methods to obtain single-enantiomer drugs mainly involve asymmetric synthesis and racemic resolution. Asymmetric synthesis includes fermentation, chiral source synthesis and asymmetric catalytic synthesis (Schuur et al. 2011). Although asymmetric synthesis has made impressive progress, racemic resolution is still the most common way in industry production (Ghanem and Aboul-Enein 2004). A series of chiral resolution methods have been developed, such as membrane separation (Weng et al. 2015), crystallization (Vetter et al. 2015), chromatography (Shen and Okamoto 2015), and enantioselective liquid–liquid extraction (ELLE) (Tang et al. 2011). However, these methods have some shortages, such as low capacity and high cost of chromatography (Lv et al. 2015), low yield and versatility of crystallization (Wacharineantar et al. 2010). Compared with above-mentioned methods, enzymatic resolution possesses the advantages of high selectivity, friendly to the environment and continuous production by coupling technology (Saric et al. 2011), and it has been widely applied in pharmaceutical industry.

Lipase (Ec 3.1.1.3) belongs to carboxyl ester hydrolase, which is employed as biocatalyst for various reactions such as acylation (Li et al. 2012), esterification (Siodmiak et al. 2015), transesterification (Miyazawa and Iguchi 2013) and hydrolysis (Leśniarek et al. 2018), etc. For example, lipase from *Candida rugosa,* lipase from *Pseudomonas cepacia* and lipase from *Aspergillus terreus*, can catalyze hydrolysis of racemic arylpropionic acid drugs (Yilmaz et al. 2011; Zhang et al. 2019; Hu et al. 2015). The preparation of optically pure compounds by lipase-catalyzed has recently become a major research field due to excellent catalytic activity and high stereoselectivity of lipase.

2-Arylpropionic acid is an important class of anti-inflammatory drugs (NSAIDs) with antipyretic and analgesic effects. 2-(4-Hydroxyphenyl)propionic acid (2-HPPA) is an intermediate of 2-arylpropionic acid drugs. Usually, two enantiomers of NSAIDs differ greatly in their pharmacological activities (Gilani et al. 2017), thus the separation of these drugs has important research significance. There are many publications that have reported the separation and synthesis of 2-arylpropionic acid (Neumann et al. 2010; Ye et al. 2010). Tong et al. have reported the separation of (±)-2-HPPA by HPLC (Tong et al. 2016), while enzymatic resolution has not been applied. In this paper, optically pure (*R*)-(−)-2-HPPA was obtained by lipase-catalyzed

✉ Kewen Tang
tangkewen@sina.com

[1] Department of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang 414006, Hunan, China

Published online: 25 April 2019

hydrolysis of 2-HPPAEE enantiomers. Effects of different reaction conditions on lipase activity and enantioselectivity were investigated. Under the optimal conditions, 2-HPPAEE enantiomers could be separated and high purity of (*R*)-(−)-2-HPPA was obtained.

Experimental

Enzymes

Lipozyme from *Rhizomucor miehei* (Lipozyme RM, 250 IUN/g), lipase from *Rhizopus niveus* (RNL, 2500 U/g) and lipase from porcine pancreas (PPL, 20,000 U/mg) were purchased from Sigma-Aldrich company (USA). Novozym 40086 is lipase from *Aspergillus oryzae* (275 IUN/g), Lipozyme TL IM is lipase from *Thermomyces lanuginosus* (250 IUN/g) and CALA is lipase A from *Candida antarctica* (6000 LU/g), which were purchased from Novozymes Biopharma DK A/S (Denmark). Lipase AK is lipase from *Pseudomonas fluorescens* (20,000 U/g) and Lipase AYS (30,000 U/g) is lipase from *Candida rugosa*, which were obtained from Amano Pharmaceutical company (Nagoya, Japan).

Chemicals

Sulfobutylether-β-cyclodextrin (SBE-β-CD) was obtained from Shandong Zhiyuan Biotechnology Co., Ltd (Shandong, China). (±)-2-HPPA was obtained from Xianju Pharmaceutical Co., Ltd (Zhejiang, China). 2-HPPAEE was prepared in the laboratory. Solvent for chromatography was of HPLC grade. All other reagents were analytical grade and obtained from different commercial suppliers.

HPLC analysis

The concentrations of resolution products, (*S*)-(+)-2-HPPA and (*R*)-(−)-2-HPPA were analyzed on reversed-phase column of ODS-3 filled with silica gel (250 mm × 4.6 mm i.d., 5 μm) by HPLC (waters e2695, Waters Corporation, USA). UV detection wavelength was set at 230 nm. The mobile phase was composed of methanol and aqueous solution (containing 25 mmol/L SBE-β-CD and 0.5% glacial acetic acid) volume ratio of 15:85 (pH 3.50, adjusted with triethylamine). The flow rate of the mobile phase was kept at 1.0 mL/min. The injection volume was 10 μL. The retention time of (*S*)-(+)-2-HPPA, (*R*)-(−)-2-HPPA, (*S*)-(+)-2-HPPAEE and (*R*)-(−)-2-HPPAEE were 10.87, 12.96, 95.38, and 99.91 min, respectively. This analytical method was adjusted by the literature (Tong et al. 2016).

The enantioselectivity (*E*) is determined by the conversion of 2-HPPAEE (*c*, %) and the enantiomeric excess of the product (ee_p, %).

$$ee_p = \frac{[(-)\text{-acid}] - [(+)\text{-acid}]}{[(-) - \text{acid}] + [(+)\text{-acid}]} \times 100\%, \quad (1)$$

$$c = \frac{([(-)\text{-acid}] + [(+)\text{-acid}])V}{n_{2-\text{HPPAEE},0}} \times 100\%, \quad (2)$$

$$E = \frac{\ln[1 - c(1 + ee_p)]}{\ln[1 - c(1 - ee_p)]}, \quad (3)$$

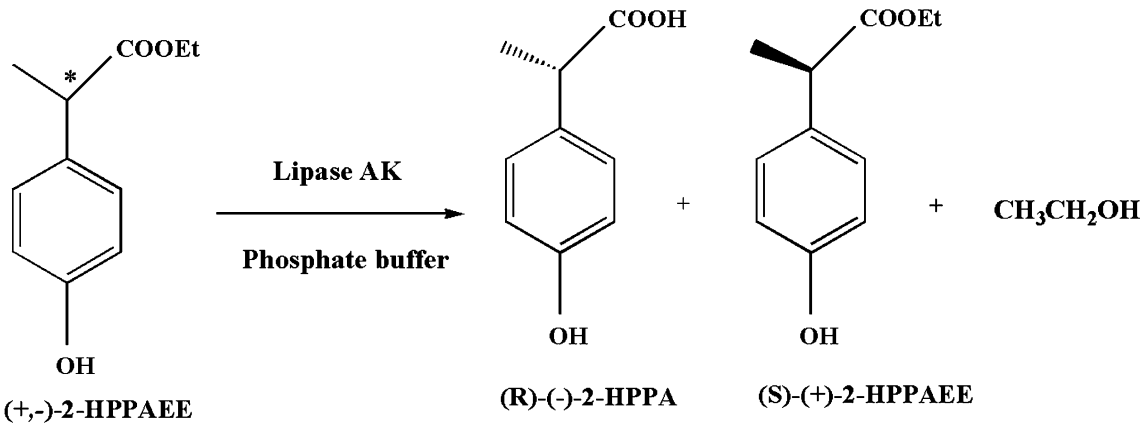
where [(−)-acid] and [(+)-acid] represent the concentration of (−)-2-HPPA and (+)-2-HPPA, respectively; *V* is the volume of reaction mixture; $n_{2\text{-HPPAEE},0}$ is the initial amount of 2-HPPAEE (mmol).

Synthesis of 2-HPPAEE

2-HPPAEE was synthesized by esterification of commercially available (±)-2-HPPA and ethanol. (±)-2-HPPA (3.32 g, 20 mmol) and p-toluenesulfonic acid (0.86 g, 5 mmol) were dissolved in ethanol (29 mL, 800 mmol). The mixture was added to a round-bottomed flask equipped with a condenser tube and stirred for 12 h at 80 °C in an electrothermally heated oil bath. Reaction was monitored with TLC plate until the esterification reaction was terminated. To remove the remaining substrate and p-toluenesulfonic acid, the saturated sodium bicarbonate solution was applied to wash-reactive mixture. The mixture was extracted with ethyl acetate and then washed with deionized water to neutral. Anhydrous sodium sulfate was used to dry the separated organic phase and then concentrated by distillation under reduced pressure (40 °C, 20 kPa). The spectroscopic data of product are in accordance with literature (Ushiyama and Furuya 1989). Finally, the racemic 2-HPPAEE (yield, ≥ 90%; purity, ≥ 98%) was obtained as slight yellow oil.

Enzymatic hydrolysis of (±)-2-HPPAEE

The enzymatic hydrolysis reaction operated in a heated magnetic stirred reactor (IKA RCT basic, Germany) equipped with 25 mL schlenk tube. The agitation speed was kept 400 rpm. A certain amount of (±)-2-HPPAEE and lipase were added in 2 mL of 0.1 mol/L dibasic sodium phosphate buffer to initialize the reaction. The hydrolysis reaction is illustrated in Scheme 1. After the reaction was completed, 1 mL of acetonitrile was added to the reaction solution to dilute and dissolve all the substrates and products. Then, 0.45 μm microporous membrane was used to filter the reaction mixture. The concentrations of 2-HPPA enantiomers



Scheme 1 Lipase-catalyzed enantioselective hydrolysis of (±)-2-HPPAEE in buffer medium

Table 1 Results of hydrolysis of (±)-2-HPPAEE catalyzed by different lipases

Enzyme species	Source	ee_p (%)	*c* (%)	*E*
Lipase AYS	*Candida rugosa*	85	4	13
CALA	*Candida antarctica*	29	21	2
Lipase AK	*Pseudomonas fluorescens*	98	34	164
Lipozyme RM	*Rhizomucor miehei*	9	4	2
Novozym 40086	*Aspergillus oryzae*	40	4	1
Lipozyme TL	*Thermomyces lanuginosus*	26	2	2
PPL	*Porcine pancreatic*	–	–	–
RNL	*Rhizopus niveus*	–	–	–

Conditions: reaction temperature 45 °C, pH 6.50, 10 mg lipase, 0.04 mmol (±)-2-HPPAEE, 24 h, and reaction volume 2 mL

were analyzed by HPLC. All the experiments of this paper were repeated three times under identical conditions and the precision level of the replicated values was within ± 3%.

Results and discussion

Selection of various lipases

The catalytic efficiency of different lipases may differ greatly in enantioselective hydrolysis of racemic 2-HPPAEE. Therefore, screening of lipases is necessary to prioritize studies of other reaction conditions. The stereoselectivity and activity of nine lipases were evaluated by the conversion and enantiomeric excess under identical conditions (Table 1). Table 1 shows the results of conversion of substrate (*c*), enantiomeric excess of product (ee_p) and enantioselectivity (*E*) of each lipase after 24 h of reaction. Lipozyme RM, PPL and RNL are essentially unable to catalyze the hydrolysis of (±)-2-HPPAEE. Lipase AYS, Novozym 40086 and Lipozyme TL have a certain enantioselectivity, while the catalytic activity of them is not high. Lipase AK showed the highest E value (164) and ee_p (> 98%). This may be because the substrate specificity of lipase determines that lipase AK prefers to form a complex with (*R*)-(−)-2-HPPAEE compared to other lipases. Therefore, lipase AK was chosen as the best biocatalyst for all further experiments based on the results of activity and selectivity.

Effect of temperature

As we all know, in biocatalytic stereochemical reaction, temperature has a great impact on enzyme activity, enantioselectivity and reaction rate. Too low temperature will inhibit enzyme activity, while too high temperature will make the enzyme lose activity. Therefore, it is necessary to find the optimum temperature of lipase AK-catalyzed hydrolysis of (±)-2-HPPAEE reaction. As shown in Fig. 1a, b, *c*, ee_s, ee_p and *E* increased rapidly with the increase of temperature (≤ 45 °C), and then they decreased with a further increase of temperature (≥ 45 °C). Therefore, the maximum *c* (26%), ee_s (35%), ee_p (98%) and *E* (161) are obtained at 45 °C. The decrease of *c*, ee_s, ee_p and *E* in high temperature might be explained by the reason that high temperature could damage the three-dimensional structure of lipase AK and lead to the loss of the activity. Furthermore, the excessive high temperature could result in the conformational change of the active site (Dong et al. 2010). When the temperature decreases (≤ 45 °C), the energy barrier of two enantiomers decreases, resulting in the reduction of enantioselectivity. On the other hand, the collision chance between enzyme and substrate molecules decreased which might be detrimental to form enzyme–substrate complexes and then the reaction rate was reduced at lower temperature. To maintain the activity and selectivity of the lipase, an appropriate temperature was needed. Therefore, the reaction temperature was set at 45 °C.

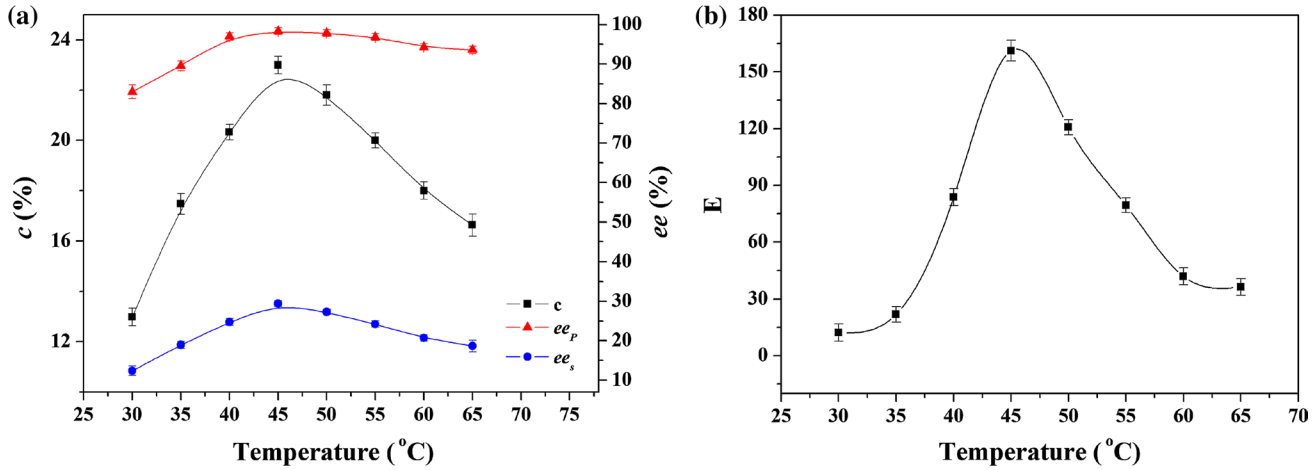


Fig. 1 Effect of temperature on c, ee_s, ee_p and E. Conditions: pH 5.5, 10 mg lipase AK, 0.04 mmol (±)-2-HPPAEE, reaction time 6 h, and reaction volume 2 mL

Effect of pH

The pH of buffer solution has a great effect on lipase-catalyzed hydrolysis reaction. Generally, lipases have an optimal pH, and higher and lower values can lead to partial inactivation. Therefore, it is very important to find the optimum pH for lipase-catalyzed hydrolysis of (±)-2-HPPAEE reaction. Figure 2a, b depicts the influence of pH on c, ee_s, ee_p and E. It is observed from Fig. 2 that c, ee_p and E increased with increasing the pH of buffer solution ranging from 4.0 to 5.5. With a further increase of pH, ee_s, c and E decreased rapidly, while ee_p decreased slightly. The maximum values of c (22%), ee_s (28%), and E (161) were obtained at pH 5.5. Lipases have been reported to possess similar "catalytic triplet" residues constructed from His, Ser and another amino acid residue (Arpigny and Jaeger 1999). In catalytic process, the catalytic triad is converted into a tetrahedral transition state by hydrogen bonding with the substrate, which is a key step in determining the reaction rate and selectivity. Since lipase is a catalytically active biomacromolecule, the tertiary structure of the enzyme is destroyed at too high or too low pH, which leads to the loss of catalytic activity (Cho et al. 2011; Dong et al. 2010). Meanwhile, due to the destruction of the three-dimensional structure of the enzyme, the active site was reduced and then the initial reaction rate and enantioselectivity were decreased. Similar results have been reported in enantioselective hydrolysis of (*R*/*S*)-naproxen methyl ester using lipase catalyst (Erdemir and Yilmaz 2012). Based on the above results, pH of 5.5 was selected as the optimum pH.

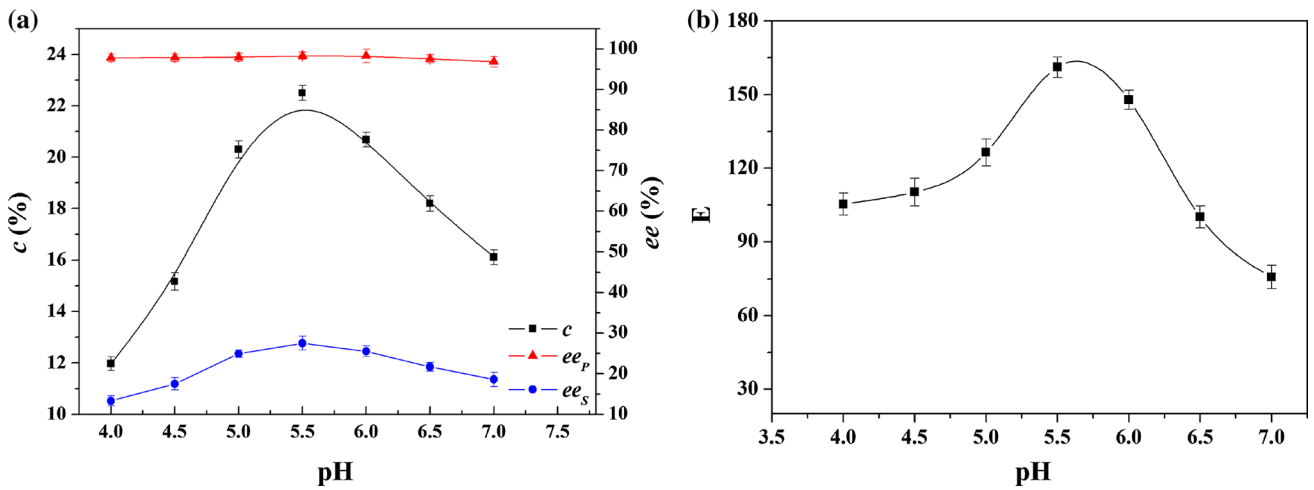


Fig. 2 Effect of pH on c, ee_s, ee_p and E. Conditions: reaction temperature 45 °C, 10 mg lipase AK, 0.04 mmol (±)-2-HPPAEE, reaction time 6 h, and reaction volume 2 mL

Effect of lipase AK dosage

In lipase-catalyzed stereochemical reaction, increasing the dosage of enzyme used can shorten the reaction time required for the same conversion, but at the same time it will increase the production cost of the process. Hence, it is needed to determine an appropriate enzyme dosage. Figure 3a, b shows the effect of lipase AK dosage on *c*, ee_s, ee_p and *E*. As shown in Fig. 3a, *c* and ee_s increase rapidly with increasing the lipase AK dosage in the buffer containing 0.04 mmol of the dosage of substrate. When the enzyme dosage reaches 10 mg, they remain unchanged with a further increase of the lipase AK dosage. In addition, ee_p has no significant effect as the amount of enzyme increases. From Fig. 3b, *E* increases with the increase of enzyme dosage ($\leq$10 mg), and further increase of enzyme dosage will lead to a significant downward trend ($\geq$10 mg). The results show that *E* is decreased from 330 to 266 with the increase of enzyme dosage ($\geq$10 mg). This may be attributed to the use of free enzymes as catalysts to hydrolyze HPPAEE in phosphate buffers. The enzyme is appears easily in an intense aggregation tendency in such a reaction system (Palomo et al. 2003; Marciello et al. 2012). The *E* decreased with the increase of enzyme dosage, which may be due to the mutual aggregation of enzyme molecules at a higher concentration. This result is consistent with that has been reported in the literature (Takaç and Mutlu 2007). Therefore, lipase AK dosage of 10 mg was selected with the substrate dosage of 0.04 mmol.

Effect of substrate dosage

The dosage of substrate in the enzymatic reaction is a key factor affecting its potential application. Effects of (±)-2-HPPAEE (substrate) dosage on *c*, ee_s and ee_p were investigated. As shown in Fig. 4, ee of product (ee_p) is very high (approximately 98%) and can keep unchanged in the tested dosage of substrate. The conversion is very high (approximately 50%) with a relatively low substrate dosage ($\leq$0.04 mmol) and then decreases with increasing the substrate dosage ($\geq$0.04 mmol). The increase of substrate dosage also causes the decrease of ee_s. The above phenomenon may be explained as follows: when the substrate dosage is low ($\leq$0.04 mmol), enzyme only binds to (*R*)-(−)-HPPAEE to form an enzyme–substrate complex, which leads to high ee of product. When the substrate dosage is increased ($\geq$0.04 mmol), an inhibition occurs, which leads to the reduction of conversion rate and ee_s (Takaç and Mutlu 2007). Meanwhile, to obtain excellent conversion and ee_s,

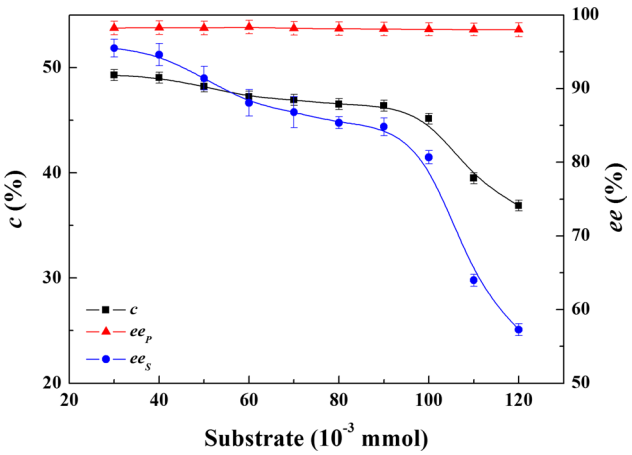


Fig. 4 Effect of substrate dosage on *c*, ee_s and ee_p. Conditions: reaction temperature 45 °C, pH 5.5, 10 mg lipase AK, reaction time 40 h and reaction volume 2 mL

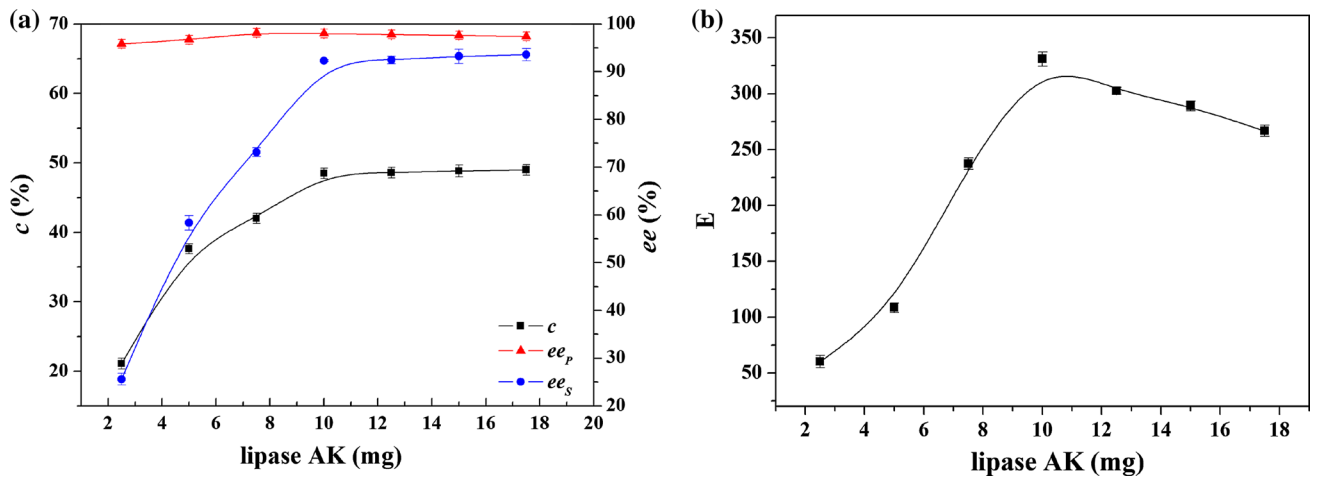


Fig. 3 Effect of the dosage of lipase AK on *c*, ee_s, ee_p and *E*. Conditions: reaction temperature 45 °C, pH 5.5, 0.04 mmol (±)-2-HPPAEE, reaction time 40 h and reaction volume 2 mL

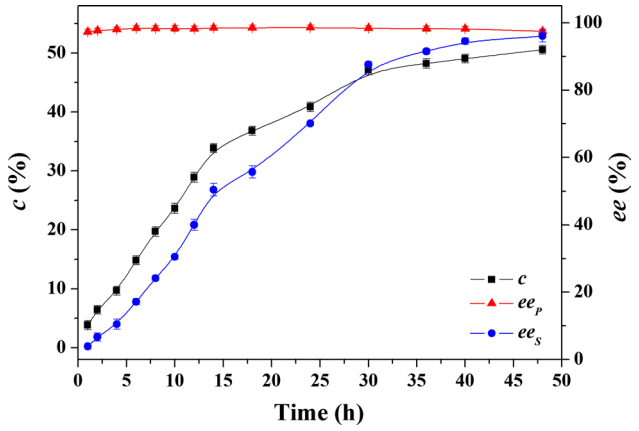


Fig. 5 Effect of reaction time on c, ee_s and ee_p. Conditions: reaction temperature 45 °C, pH 5.5, 10 mg lipase AK, 0.04 mmol (±)-2-HPPAEE and reaction volume 2 mL

the enzyme loading also needs to be increased. But commercial lipases are relatively expensive, the economic benefits are reduced. Therefore, considering the above reasons, substrate dosage of 0.04 mmol in 2 mL of reaction system was selected.

Process curve of enantioselective hydrolysis of (±)-2-HPPAEE

Reaction time is one of the important factors guiding industrial applications. When enzyme is at maximum activity, the required optimum reaction time will be reduced. Therefore, influences of reaction time on c, ee_s and ee_p were investigated. From Fig. 5, c and ee_s increased rapidly with the reaction time increase ($\leq$40 h) and then increased slowly ($\geq$40 h), while the change in ee_p is small. It might be because the reaction is highly enantioselective and only one enantiomer of HPPAEE is hydrolyzed, at around 50% conversion the reaction gets slower because in the mixture there is little amount of fast-reacting enantiomer and a lot of slow-reacting enantiomers. To meet the ee_p value of 98% and c of 49%, the reaction time required was approximately 40 h.

Application

The optimal conditions were determined by the above experiment's results, involving temperature of 45 °C, pH of 5.5, 10 mg lipase AK dosage, 0.04 mmol substrate dosage and 40 h of reaction time. Enantioselective hydrolysis of HPPAEE enantiomers were carried out by lipase catalysis under these optimal conditions. The purity of racemic 2-HPPA and obtained products were analyzed through HPLC. Figure 6a shows the chromatograms of racemic 2-HPPA. Figure 6b shows the chromatograms of the products of hydrolysis of (±)-2-HPPAEE, indicating that high purity of 98% is obtained.

Conclusions

Stereoselective lipase-catalyzed hydrolysis of 2-HPPAEE enantiomers was investigated to obtain (*R*)-(−)-2-HPPA. Lipase AK was selected as the most efficient catalyst. The effects of some important process parameters, including temperature, pH, dosage of lipase AK, dosage of substrate and reaction time were studied. It could be concluded that the enantiomeric excess of product mainly depends on the reaction temperature. The conversion of substrate primarily depends on temperature, pH, dosage of lipase AK, reaction time and dosage of substrate. The optimal conditions were achieved, including temperature of 45 °C, pH of 5.5, 10 mg lipase AK dosage, 0.04 mmol substrate dosage and reaction time of 40 h, where the conversion could reach up to 49% and the enantiomeric excess of product could reach up to 98%. A simple and feasible method is provided to obtain optically pure (*R*)-(−)-2-HPPA by this work.

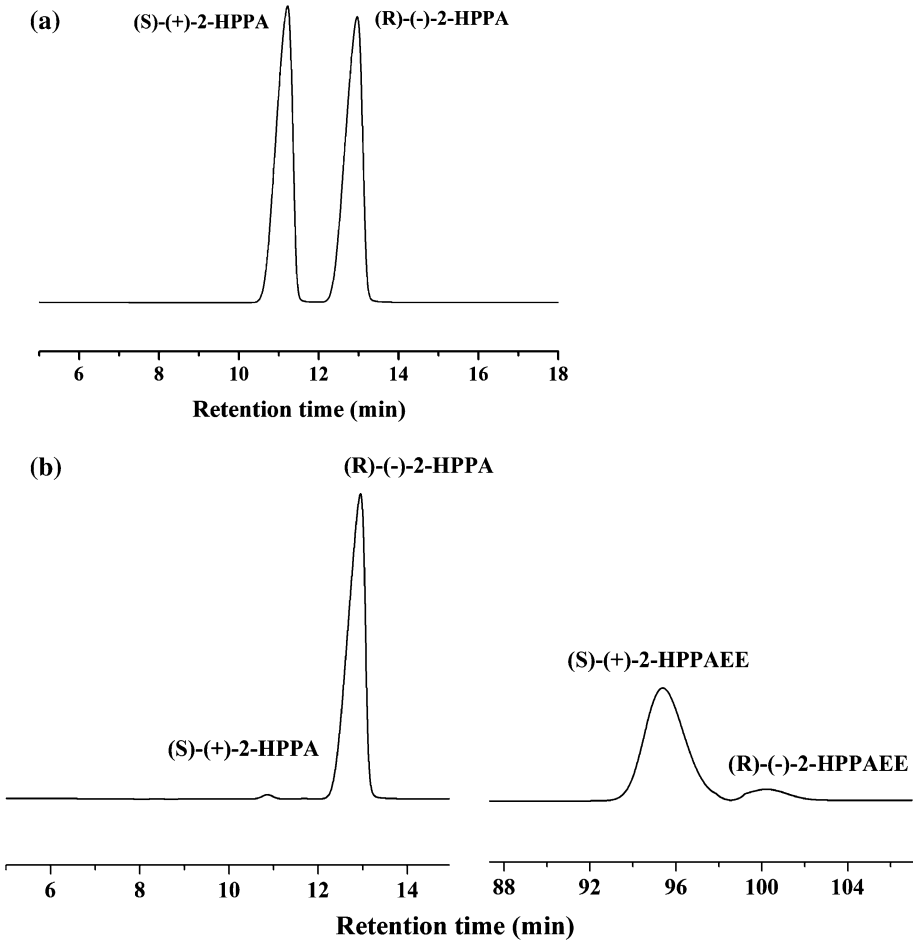


Fig. 6 HPLC chromatograms. **a** Racemate of (±)-2-HPPA, **b** products of hydrolysis of (±)-2-HPPAEE

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant numbers, 21676077).

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

References

Arpigny JL, Jaeger KE (1999) Bacterial lipolytic enzymes: classification and properties. Biochem J 343:177–183. https://doi.org/10.1042/bj3430177

Cho SH, Wang PY, Tsai SW (2011) Lipase-catalyzed hydrolytic resolution of (+,−)-3-hydroxy-3-phenylpropionates in biphasic media. J Taiwan Inst Chem E 42:408–412. https://doi.org/10.1016/j.jtice.2010.09.005

Dong HP, Wang YJ, Zheng YG (2010) Enantioselective hydrolysis of diethyl 3-hydroxyglutarate to ethyl (S)-3-hydroxyglutarate by immobilized *Candida antarctica* lipase. J Mol Catal B-Enzym 66:90–94. https://doi.org/10.1016/j.molcatb.2010.03.009

Erdemir S, Yilmaz M (2012) Catalytic effect of calix [n] arene based sol–gel encapsulated or covalent immobilized lipases on enantioselective hydrolysis of (R/S)-naproxen methyl ester. J Incl Phenom Macro Chem 72:189–196. https://doi.org/10.1007/s10847-011-9962-1

Ghanem A, Aboul-Enein HY (2004) Lipase-mediated chiral resolution of racemates in organic solvents. Tetrahedron Asymmetry 15:3331–3351. https://doi.org/10.1016/j.tetasy.2004.09.019

Gilani SL, Najafpour GD, Heydarzadeh HD, Moghadamnia A (2017) Enantioselective synthesis of (S)-naproxen using immobilized lipase on chitosan beads. Chirality 29:304–314. https://doi.org/10.1002/chir.22689

Hu C, Wang N, Zhang W et al (2015) Immobilization of Aspergillus terreus lipase in self-assembled hollow nanospheres for enantioselective hydrolysis of ketoprofen vinyl ester. J Biotechnol 194:12–18. https://doi.org/10.1016/j.jbiotec.2014.11.032

Leśniarek A, Gładkowski W, Chojnacka A (2018) Application of Lecitase® Ultra-catalyzed Hydrolysis to the Kinetic resolution of (E)-4-phenylbut-3-en-2-yl esters. Catalysts 8:423. https://doi.org/10.3390/catal8100423

Li N, Hu SB, Feng GY (2012) Resolution of 2-nitroalcohols by Burkholderia cepacia lipase-catalyzed enantioselective acylation. Biotechnol Lett 34:153–158. https://doi.org/10.1007/s10529-011-0754-x

Lv CG, Jia GF, Zhu WT et al (2015) Enantiomeric resolution of new triazole compounds by high-performance liquid chromatography. J Sep Sci 30:344–351. https://doi.org/10.1002/jssc.200600282

Marciello M, Filice M, Palomo JM (2012) Different strategies to enhance the activity of lipase catalysts. Catal Sci Technol 2:1531–1543. https://doi.org/10.1039/c2cy20125a

Miyazawa T, Iguchi W (2013) Long-chain ethers as solvents can amplify the enantioselectivity of theCarica papayalipase-catalyzed transesterification of 2-(substituted phenoxy)propanoic acid esters. Biotechnol Lett 35:1639–1643. https://doi.org/10.1007/s10529-013-1247-x

Neumann H, Brennführer A, Beller M (2010) An efficient and practical sequential one-pot synthesis of suprofen, ketoprofen and other 2-arylpropionic acids. Adv Synth Catal 350:2437–2442. https://doi.org/10.1002/adsc.200800415

Palomo JM, Fuentes M, Fernández-Lorente G, Mateo C, Guisan JM, Fernández-Lafuente R (2003) General trend of lipase to self-assemble giving bimolecular aggregates greatly modifies the enzyme functionality. Biomacromolecules 4:1–6. https://doi.org/10.1021/bm025729+

Saric M, van der Wielen LAM, Straathof AJJ (2011) Theoretical performance of countercurrent reactors for production of enantiopure compounds. Chem Eng Sci 66:510–518. https://doi.org/10.1016/j.ces.2010.11.020

Schuur B, Verkuijl BJ, Minnaard AJ, de Vries JG, Heeres HJ, Feringa BL (2011) Chiral separation by enantioselective liquid-liquid extraction. Org Biomol Chem 9:36–51. https://doi.org/10.1039/c0ob00610f

Shen J, Okamoto Y (2015) Efficient separation of enantiomers using stereoregular chiral polymers. Chem Rev 116:1094–1138. https://doi.org/10.1021/acs.chemrev.5b00317

Siodmiak T, Mangelings D, Vander Heyden Y, Ziegler-Borowska M, Marszall MP (2015) High enantioselective Novozym 435-catalyzed esterification of (R,S)-flurbiprofen monitored with a chiral stationary phase. Appl Biochem Biotechnol 175:2769–2785. https://doi.org/10.1007/s12010-014-1455-4

Takaç S, Mutlu D (2007) A parametric study on biphasic medium conditions for the enantioselective production of naproxen by Candida rugosa lipase. Appl Biochem Biotechnol 141:15–26. https://doi.org/10.1007/s12010-007-9207-3

Tang KW, Zhang PL, Pan C (2011) Experimental and simulation on enantioselective extraction in centrifugal contactor separators. AIChE J 57:3027–3036. https://doi.org/10.1002/aic.14004

Tong S, Wang X, Lu M, Xiong Q, Wang Q, Yan J (2016) Enantioseparation of 2-(substituted phenyl) propanoic acids by high-speed countercurrent chromatography and investigation of the influence of substituents in enantiorecognition. J Sep Sci 39:1567–1573. https://doi.org/10.1002/jssc.201600171

Ushiyama M, Furuya T (1989) Biotransformation of (RS)-tropic acid in suspension cultures of *Coffea arabica*, *Datura innoxia*, *Eucalyptus perriniana* and *Nicotiana tabacum*. Phytochemistry. https://doi.org/10.1016/S0031-9422(00)97979-2

Vetter T, Burcham CL, Doherty MF (2015) Separation of conglomerate forming enantiomers using a novel continuous preferential crystallization process. AIChE J 61:2810–2823. https://doi.org/10.1002/aic.14934

Wacharineantar S, Levilain G, Dupray V et al (2010) Resolution of (±)-Imeglimin-2,4-dichlorophenylacetate methanol solvate by preferential crystallization. Org Process Res Dev 14:1358–1363. https://doi.org/10.1021/op100173r

Weng X, Baez JE, Khiterer M, Hoe MY, Bao Z, Shea KJ (2015) Chiral polymers of intrinsic microporosity: selective membrane permeation of enantiomers. Angew Chem Int Ed 54:11214–11218. https://doi.org/10.1002/ange.201504934

Ye J, Yu W, Chen G et al (2010) Enantiomeric separation of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs by HPLC with hydroxypropyl-β-cyclodextrin as chiral mobile phase additive. Biomed Chromatogr 24:799–807. https://doi.org/10.1002/bmc.1365

Yilmaz E, Can K, Sezgin M et al (2011) Immobilization of Candida rugosa lipase on glass beads for enantioselective hydrolysis of racemic Naproxen methyl ester. Bioresour Technol 102:499–506. https://doi.org/10.1016/j.biortech.2010.08.083

Zhang PL, Cheng Q, Xu W et al (2019) Enzymatic enantioselective hydrolysis of 2-(3-chlorophenyl) propionic acid ester enhanced by PEG: experiment and optimization. Ind Eng Chem Res 57:11246–11256. https://doi.org/10.1021/acs.iecr.8b02377