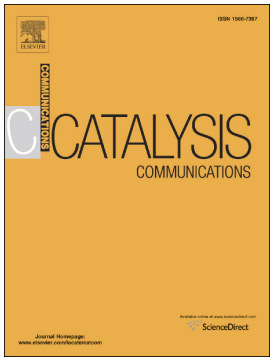

Asymmetric hydrolysis of styrene oxide by PvEH2, a novel Phaseolus vulgaris epoxide hydrolase with extremely high enantioselectivity and regioselectivity

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Asymmetric hydrolysis of styrene oxide by *Pv*EH2, a novel *Phaseolus vulgaris* epoxide hydrolase with extremely high enantioselectivity and regioselectivity

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ABSTRACT

A novel EH from *Phaseolus vulgaris*, *Pv*EH2, was discovered based on the computer-aided analysis, and its encoding gene was cloned and expressed in *E. coli* Rossetta(DE3). The substrate spectrum of recombinant (re) *Pv*EH2 was assayed, among which the enantiomeric ratio of re*Pv*EH2 towards racemic styrene oxide (*rac*-**1a**) was more than 200, while its regioselectivity coefficients, α_S and β_R, towards (*S*)- and (*R*)-**1a** were 99.1 and 69.8%, respectively. The asymmetric hydrolysis of 20 mM *rac*-**1a** by re*Pv*EH2-expressing whole cells was performed at 25 °C, retaining (*R*)-**1a** with > 99.5% ee_s and 49.4% yield and producing (*R*)-phenyl-1,2-ethanediol (**1b**) with 96.2% ee_p and 49.7% yield in 40 min.

Keywords: Epoxide hydrolase; *Phaseolus vulgaris*; Styrene oxide; Enantioselectivity; Regioselectivity; Asymmetric hydrolysis

1. Introduction

Chiral epoxides and their corresponding vicinal diols are extensively used in the synthesis of fine chemicals, pharmaceuticals and agrochemicals [1]. Among these versatile building blocks, chiral **1a** and its hydrolysate, (*R*)- or (*S*)-**1b**, are important synthons for producing anticancer agents, adrenergic receptor agonists and kinase inhibitors [2]. Recently, several chemical asymmetric syntheses of chiral **1a** and **1b** have been reported, such as Jacobsen epoxidation and Sharpless dihydroxylation [3]. However, catalysts having potential pollution were required in these methods, and the enantiomeric excess (*ee*) values and/or yields of products were unsatisfactory in some cases [4].

Epoxide hydrolases (EHs, EC 3.3.2.3) stereoselectively catalyze the addition of water to epoxide ring. With the green wave of global industries, the biotransformation by EHs, an environmental-friendly way, has received much attention [5]. To date, EHs from plants, microorganisms, mammals and invertebrates have been discovered and characterized, but few EHs exhibited desirable enantioselectivity and regioselectivity [6,7]. For example, *Au*EH2 from *Aspergillus usamii* displayed a modest preference for (*R*)-**1a** in the resolution of *rac*-**1a**, retaining (*S*)-**1a** with 99.2% ee_s, but only 38% yield, lower than its theoretical yield of 50% [8]. As to plant EHs, only one from *Solanum tuberosum*, *St*EH, had high enantiomeric ratio (*E*) value of 30. Thus, *St*EH highly preferentially hydrolyzed (*S*)-**1a**, retaining (*R*)-**1a** with nearly 100% ee_s. Additionally, owing to its high and complementary regioselectivity towards (*S*)- and (*R*)-**1a**, an ee_p of (*R*)-**1b** reached 93% in 90 min. However, its enantiopurity decreased to 89% ee_p when the reaction time was prolonged to 120 min [9,10].

In this work, using the *Phaseolus vulgaris* EH1 (*Pv*EH1, GenBank: AKJ75509) [11] as the template, a novel EH also from *P. vulgaris*, *Pv*EH2, was selected based on the homology sequence search and multiple sequence alignment. A *Pv*EH2-encoding gene *pveh2* was amplified by RT-PCR from *P. vulgaris* total RNA, and expressed in *Escherichia coli* Rosetta(DE3). The *E* values of re*Pv*EH2 towards ten *rac*-epoxides (Fig. 1) and its α_S and β_R values towards (*S*)- and (*R*)-epoxides were determined, respectively. Then, the asymmetric hydrolysis of *rac*-**1a** was conducted by the whole cells expressing re*Pv*EH2 to simultaneously prepare (*R*)-**1a** and (*R*)-**1b**.

2. Experimental

2.1. Materials

P. vulgaris was purchased from the local supermarket for total RNA extraction. All enzymes and kits used for gene cloning were from TaKaRa (Dalian, China), while pET-28a(+) and *E. coli* Rosetta(DE3) (Novagen, Madison, WI) were used for expression of *pveh2*. *E. coli* Rosetta(DE3) and its recombinants were cultured in the

LB medium and induced by IPTG. *Rac*-**1a**, **7a**, (*S*)-**1a**, (*R*)-**1a**, (*S*)-**1b** and (*R*)-**1b** were products of TCI (Tokyo, Japan), while *rac*-**6a** was purchased from Energy (Shanghai, China). *Rac*-**2a**–**5a** and **8a**–**10a** were synthesized in our lab.

2.2. Database search and sequence analysis

Sequences of hypothetical proteins with unknown functions, sharing identities more than 75% with that of *Pv*EH1 (AKJ75509), were searched at NCBI (http://www.ncbi.nlm.nih.gov/) by BLAST server. Among them, a hypothetical one (XP_007147001) also from *P. vulgaris*, *Pv*EH2, was selected. Based on the multiple sequence alignment with five plant EHs by ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/), *Pv*EH2 was confirmed as the study object in this work.

2.3. Cloning and expression of pveh2

The first-strand cDNA was obtained from *P. vulgaris* total RNA by reverse transcription using Oligo dT-Adaptor primer in an RNA PCR kit. Then, the *Pv*EH2-encoding gene (*pveh2*), flanked by *Nde* I and *Xho* I sites, was PCR-amplified using a pair of primers *Pv*2-F and *Pv*2-R (Table S1), and ligated with pET-28a(+), followed by DNA sequencing. The correct recombinant expression plasmid pET-28a-*pveh2* was transformed into *E. coli* Rosetta(DE3), forming a recombinant *E. coli* (*E. coli*/*pveh2*). *E. coli* Rosetta(DE3) transformed with pET-28a(+) (*E. coli*/pET-28a) was used as the negative control.

A single colony of *E. coli*/*pveh2* or *E. coli*/pET-28a was inoculated into LB medium containing 100 μg/mL kanamycin, and cultured at 37 °C overnight as the seed culture. Then, fresh LB medium was inoculated with 2% (v/v) seed culture, and cultured until OD_{600} reached 0.6–0.8. After induction by 0.2 mM IPTG at 20 °C for 9 h, the cells were collected and resuspended in Na_2HPO_4–NaH_2PO_4 buffer (50 mM, pH 7.2). The resulting cell suspension (100 mg wet cell/mL) was used as the catalyst.

2.4. Protein and enzyme activity assay

The expression of re*Pv*EH2 was analyzed by SDS-PAGE. Its apparent molecular weight was estimated by Quantity One software based on the standard proteins. EH activity was measured by high-performance liquid chromatography (HPLC) as described previously [11] using an e2695 apparatus (Waters, Milford, MA) equipped with a C18 column. One unit (U) of EH activity was defined as the amount of re*Pv*EH2 generating 1 μmol **1b** per min under the assay conditions.

2.5. Stereoselectivity assay of rePvEH2

The hydrolytic reactions of *rac*-**1a**–**10a**, (*S*)- and (*R*)-**1a** were performed as follows: 1.0 mL cell suspension (100 mg/mL) was separately mixed with 1.0 mL *rac*-**1a**–**10a** (10 mM), (*S*)- and (*R*)-**1a** (5 mM) in 50 mM

Na_2HPO_4–NaH_2PO_4 buffer (pH 7.2), and then incubated at 25 °C. During the hydrolytic reaction, 50-μL samples were withdrawn at different time points, extracted with 400 μL ethyl acetate, and assayed by chiral gas chromatography (GC) using a GC-2010 apparatus (Shimadzu, Tokyo, Japan) or HPLC to calculate the ee_s of substrate, ee_p of product and conversion ratio (c).

The enantiomeric ratio (E) represents a degree of preferential hydrolysis of an enantiomer over the other one, that is, enantioselectivity [12]. The ee_s of retaining epoxide was calculated: $ee_s = [(R - S)/(R + S)] \times 100\%$, while E value: $E = \ln[(1 - c) \times (1 - ee_s)]/\ln[(1 - c) \times (1 + ee_s)]$. R and S are the concentrations of (R)- and (S)-epoxide, respectively. The regioselectivity coefficients, α_S and β_R, were related to attacks at C_α (a hindered benzylic carbon in an epoxide ring) of (S)-epoxide and at C_β (an unhindered terminal carbon) of (R)-epoxide, respectively [13]. In this work, the α_S and β_R of re*Pv*EH2 towards (S)- and (R)-**1a** were calculated based on the concentrations of (R)- and (S)-**1b**, using (S)- and (R)-**1a** as substrates, respectively [14], while the coefficients towards (S)- and (R)-**2a**–**10a** were derived from the equation: $ee_p = \alpha_S + \beta_R - 1 + [(\beta_R - \alpha_S) \times ee_s \times (1 - c)]/c$, using their racemic forms [15]. The absolute configurations of enantiomers of **2a**–**10a** and **2b**–**10b** were established by comparing their retention times with those reported previously [13,15,16].

*2.6. Asymmetric hydrolysis of rac-**1a** by whole cells*

Both 5.0 mL cell suspension (100 mg/mL) and 4.0 mL Na_2HPO_4–NaH_2PO_4 buffer were preheated at 25 °C for 5 min, and then 1.0 mL *rac*-**1a** (200 mM) was added, giving the final cell and substrate concentrations of 50 mg/mL and 20 mM, respectively. The reaction was carried out, and aliquots of 100 μL sample were drawn out at different time points in 60 min. The ee_p of (R)-**1b** was calculated: $ee_p = [(R_p - S_p)/(R_p + S_p)] \times 100\%$, while yields of ($R$)-**1a** and ($R$)-**1b**: $Y_s = [R/(R_0 + S_0)] \times 100\%$ and $Y_p = [R_p/(R_0 + S_0)] \times 100\%$. R_p and S_p are the concentrations of (R)- and (S)-**1b**, while R_0 and S_0 are the initial concentrations of (R)- and (S)-**1a** [7].

3. Results and discussion

3.1. Selection and analysis of PvEH2 sequence

Using *Pv*EH1 sequence as the template, those of six hypothetical proteins sharing over 75% identities were searched by BLAST, among which a hypothetical *Pv*EH2 shared 80.1% identity with *Pv*EH1. It was speculated that *Pv*EH2 may have EH activity owing to higher primary structure homology. Thus, this work selected *Pv*EH2 as the study object. The multiple sequence alignment of *Pv*EH2 with five plant EHs displayed that it has typical conserved motifs, identical to those of the α/β fold EH family [8,14]: HGXP, GXSmXS/T and SmXNuXSmSm, in which X, Sm and Nu represent any, small and nucleophilic residues, respectively (Fig. 2). The catalytic triad,

consisting of nucleophile Asp, general base His and charge-relay Asp or Glu, was predicted to be Asp^{101}-His^{295}-Asp^{260} in *Pv*EH2. In addition, Tyr^{150} and Tyr^{230} in *Pv*EH2 are also conserved. There is evidence that the Tyr residues play key roles in substrate binding and epoxide ring opening via formation of hydrogen bonds [17].

3.2. Cloning and expression of pveh2

An about 1.0-kb band of *pveh2* was cloned from *P. vulgaris* total RNA by RT-PCR. DNA sequencing result verified that *pveh2* is exactly 963 bp in length (containing *Nde* I and *Xho* I sites), encoding *Pv*EH2 of 316 amino acids. After *E. coli*/*pveh2* was induced by 0.2 mM IPTG at 20 °C for 9 h, the activity of re*Pv*EH2-expressing whole cells was assayed to be 8.0 U/g wet cell, while no EH activity was detected in *E. coli*/pET-28a under the same conditions. Very low EH activity was detected when the temperature during induction exceeded 25 °C, resulting in that most re*Pv*EH2 molecules were transformed into insoluble inclusion body [8]. SDS-PAGE analysis displayed that the apparent molecular weight of re*Pv*EH2 was 38.1 kDa (Fig. 3), which well matched with its theoretical one (37,898 Da).

3.3. Enantioselectivity (E value) of rePvEH2

To assay the substrate spectrum of re*Pv*EH2, ten racemic epoxides were subjected to biotransformation by *E. coli*/*pveh2* (Table 1). Except for *rac*-**2a** and **4a**, re*Pv*EH2 preferentially hydrolyzed (*S*)-enantiomers and exhibited *E* values ranging from 1.6 to > 200, indicating that the type of epoxides and position of substituents obviously affected its activity and enantioselectivity. In detail, re*Pv*EH2 exhibited the moderate enantioselectivity towards **6a**–**10a** and poor enantioselectivity towards **3a** and **5a**. Unexpectedly, the *E* value of re*Pv*EH2 towards **1a** was determined to be > 200, displaying the extremely high preference for (*S*)-**1a**. That, to some extent, implied that the great majority of (*R*)-**1a** will be retained, and that the complete hydrolysis of *rac*-**1a** needs a quite long time [4]. To our knowledge, re*Pv*EH2 displayed the highest enantioselectivity (*E* value) towards *rac*-**1a** among all EHs hitherto reported, such as *St*EH1 (*E* = 30) [9], Kau2 (*E* = 65) [15] and *Cc*EH (*E* = 8.8) [18].

3.4. Regioselectivity (α_S and β_R) of rePvEH2

The carbon (C_α or C_β) that is mainly subjected to nucleophilic attack by Asp in a catalytic triad was highly dependent on the EH-substrate pair [9]. To make a better understanding of the catalytic mechanism of re*Pv*EH2, its regioselectivity coefficients were determined (Table 1). As a result, re*Pv*EH2 attacked (*S*)-**1a**, **3a** and **5a** mainly at C_α (α_S > 90%), giving (*R*)-diols via an inversion of configuration. Simultaneously, the C_β atoms of (*R*)-**3a** and **5a** were primarily attacked (β_R > 95%), also affording (*R*)-diols via a retention of configuration. The high and complementary regioselectivity as well as low enantioselectivity towards **3a** (*E* = 1.6) and **5a** (*E* = 1.9)

indicated that re*Pv*EH2 has a potential to catalyze their enantioconvergent hydrolysis [11,13]. In the cases of **6a**–**10a,** re*Pv*EH2 displays the poor regiocomplementarity. Considering the higher *E* values towards **7a** (E = 18.9) and **10a** (E = 23.0), re*Pv*EH2 can be used for the kinetic resolution of *rac*-**7a** and **10a**.

*3.5. Preparation of both (R)-**1a** and (R)-**1b** from rac-**1a** by whole cells*

The asymmetric hydrolysis of 20 mM *rac*-**1a** was conducted by *E. coli/pveh2* whole cells at 25 °C in 60 min, and monitored at given time intervals (Fig. 4). During the reaction, the concentration of (*S*)-**1a** decreased rapidly while that of (*R*)-**1a** was nearly invariable, which resulted in the rapid increase of ee_s of (*R*)-**1a** with high yield. Additionally, owing to the high and complementary regioselectivity of re*Pv*EH2 towards two enantiomers of **1a**, the ee_p of (*R*)-**1b** kept more than 95%. As the reaction proceeded to 40 min (c = 51.0%) (Fig. S1), (*R*)-**1a** with > 99.5% ee_s and 49.4% Y_s as well as (*R*)-**1b** with 96.2% ee_p and 49.7% Y_p was obtained, simultaneously. However, (*S*)-**1a** could not be completely hydrolyzed in 6 h (c < 25%) when the concentration of *rac*-**1a** was increased to 50 mM. The reasons may be instability of EHs under the operating conditions, inhibition of substrate or product on EHs and low solubility of epoxides in water [19]. Several methods were used to overcome these problems. For examples, the directed evolution has been adopted to create new enzymes with enhanced stability and tolerance. Another approach was to reduce the inhibition via a biphasic system [20].

4. Conclusions

In this work, *Pv*EH2 was selected based on the computer-aided analysis, and its encoding gene *pveh2* was cloned and expressed in *E. coli* Rossetta(DE3). The substrate spectrum assay indicated that re*Pv*EH2 exhibited the different catalytic modes towards various epoxides. Owing to the extremely high enantioselectivity (E > 200) and regioselectivity (α_S = 99.1%) of re*Pv*EH2, both (*R*)-**1a** and (*R*)-**1b** with high ee_s and ee_p were simultaneously prepared from the asymmetric hydrolysis of *rac*-**1a** by it. More surprisingly, the Y_s of (*R*)-**1a** was quite close to its theoretical value. The obtained *Pv*EH2 not only increased the number of EHs with high enantioselectivity and regioselectivity, but also established a foundation for the study of its catalytic mechanism.

Acknowledgment

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References

[1] H.X. Jin, X.K. Ouyang, RSC Adv. 5 (2015) 92988–92994.

[2] K. Wu, H.L. Wang, L.F. Chen, H.Y. Fan, Z.Q. Zhao, D.Z. Wei, Appl. Microbiol. Biotechnol. 100 (2016) 8757–8767.

[3] Y. Genzel, A. Archelas, Q.B. Broxterman, B. Schulze, R. Furstoss, J. Org. Chem. 66 (2001) 538–543.

[4] M. Kotik, A. Archelas, R. Wohlgemuth, Curr. Org. Chem. 16 (2012) 451–482.

[5] P. Saini, D. Sareen, Mol. Biotechnol. 59 (2017) 98–116.

[6] W.J. Choi, Appl. Microbiol. Biotechnol. 84 (2009) 239–247.

[7] J.H. Woo, K.M. Kang, T.H. Kwon, N.H. Park, E.Y. Lee, J. Ind. Eng. Chem. 28 (2015) 225–228.

[8] D. Hu, C.D. Tang, B. Yang, J.C. Liu, T. Yu, C. Deng, M.C. Wu, J. Ind. Microbiol. Biotechnol. 42 (2015) 671–680.

[9] M.I. Monterde, M. Lombard, A. Archelas, A. Cronin, M. Arand, R. Furstoss, Tetrahedron: *Asymmetry* 15 (2004) 2801–2805.

[10] C. Mateo, R. Fernandez-Lafuente, A. Archelas, J.M. Guisan, R. Furstoss, Tetrahedron: *Asymmetry* 18 (2007) 1233–1238.

[11] H.H. Ye, D. Hu, X.L. Shi, M.C. Wu, C. Deng, J.F. Li, Catal. Commun. 87 (2016) 32–35.

[12] M. Kotik, P. Kyslík, Biochim. Biophys. Acta 1760 (2006) 245–252.

[13] W. Xu, J.H. Xu, J. Pan, Q. Gu, X.Y. Wu, Org. Lett. 8 (2006) 1737–1740.

[14] Q.Q. Zhu, W.H. He, X.D. Kong, L.Q. Fan, J. Zhao, S.X. Li, J.H. Xu, Appl. Microbiol. Biotechnol. 98 (2014) 207–218.

[15] M. Kotik, V. Štěpánek, M. Grulich, P. Kyslík, A. Archelas, J. Mol. Catal. B: enzym. 65 (2010) 41–48.

[16] N. Bala, S.S. Chimni, H.S. Saini, B.S. Chadha, J. Mol. Catal. B: enzym. 63 (2010) 128–134.

[17] A. Archelas, W. Zhao, B. Faure, G Iacazio, M. Kotik, Arch. Biochem. Biophys. 591 (2016) 66–75.

[18] S. Hwang, H. Hyun, B. Lee, Y. Park, C. Choi, J. Han, H. Joo, J. Microbiol. Biotechnol. 16 (2006) 32–36.

[19] S.S. Yoo, S. Park, E.Y. Lee, Biotechnol. Lett. 30 (2008) 1807–1810.

[20] P.F. Gao, A.T. Li, H.H. Lee, D.I.C. Wang, Z. Li, ACS Catal. 4 (2014) 3763–3771.

Figure captions

Fig. 1. Ten epoxides used for substrate spectrumassay. **1a**, styrene oxide; **2a**, *para*-nitrostyrene oxide; **3a**, *meta*-nitrostyrene oxide; **4a**, *para*-chlorostyrene oxide; **5a**, *meta*-chlorostyrene oxide; **6a**, benzyl glycidyl ether; **7a**, phenyl glycidyl ether; **8a**, *para*-methylphenyl glycidyl ether; **9a**, *meta*-methylphenyl glycidyl ether; **10a**, *ortho*-methylphenyl glycidyl ether.

Fig. 2. The multiple sequence alignment of six plant EHs. *Pv*EH2 (ASS33914, in this work); *Pv*EH1 (AKJ75509, 80.1% identity with *Pv*EH2); *Vr*EH1 (ADP68585, 75.5%); *Gm*EH (CAA55294, 75.3%); *Nb*EH (ACE82566, 67.8%); *St*EH (AAA81891, 57.6%). The conserved motifs of HGXP, GXSmXS/T and SmXNuXSmSm are boxed, while NC, cap and core loops are underlined. A catalytic triad (Asp^{101}-His^{295}-Asp^{260}) and both Tyr^{150} and Tyr^{230} in *Pv*EH2 are marked with stars.

Fig. 3. SDS-PAGE analysis of the expressed re*Pv*EH2. Lane M, standard proteins; lane 1, *E. coli*/pET-28a; lane 2, *E. coli/pveh2* without induction; lane 3, *E. coli/pveh2* induced by 0.2 mM IPTG

Fig. 4. (a) The process curve of hydrolytic reaction of 20 mM *rac*-**1a** by re*Pv*EH2-expressing *E. coli/pveh2* whole cells at 25 °C. Symbols: *triangle*, *c* of *rac*-**1a**; *solid square*, (*S*)-**1a** concentration; *hollow square*, (*R*)-**1a** concentration; *solid circle*, ee_p of (*R*)-**1b**; *hollow circle*, ee_s of (*R*)-**1a**. (b) A schematic diagram of asymmetric hydrolysis of *rac*-**1a** by re*Pv*EH2 into both (*R*)-**1a** and (*R*)-**1b**.

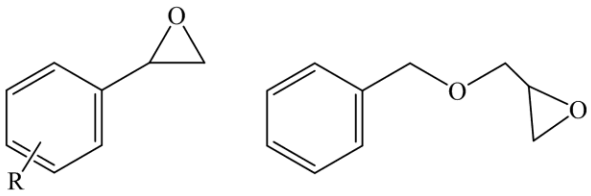
Table 1

The enantioselectivity of re*Pv*EH2 towards *rac*-**1a**–**10a** and its regioselectivity towards (*S*)- and (*R*)-**1a**–**10a**.

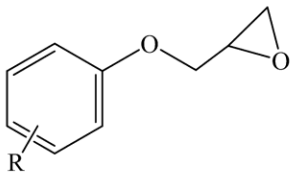
Substrate	Activity (U/g wet cell)	*E* value	α_S (%)	β_R (%)
1a	8.0	> 200	99.1	69.8
2a	ND[a]	–[b]	–	–
3a	1.3	1.6	90.3	96.4
4a	ND	–	–	–
5a	1.5	1.9	93.0	95.8
6a	2.2	8.7	23.3	87.5
7a	3.0	18.9	12.9	93.7
8a	2.4	6.2	10.7	91.1
9a	6.6	9.1	1.6	98.7
10a	4.1	23.0	4.5	96.2

[a] No detectable activity.

[b] No determination.



1-5a **6a**



7-10a

1a: R= -H
2a: R= -*p*NO_2
3a: R= -*m*NO_2
4a: R= -*p*Cl
5a: R= -*m*Cl
7a: R= -H
8a: R= -*p*CH_3
9a: R= -*m*CH_3
10a: R= -*o*CH_3

Figure 1

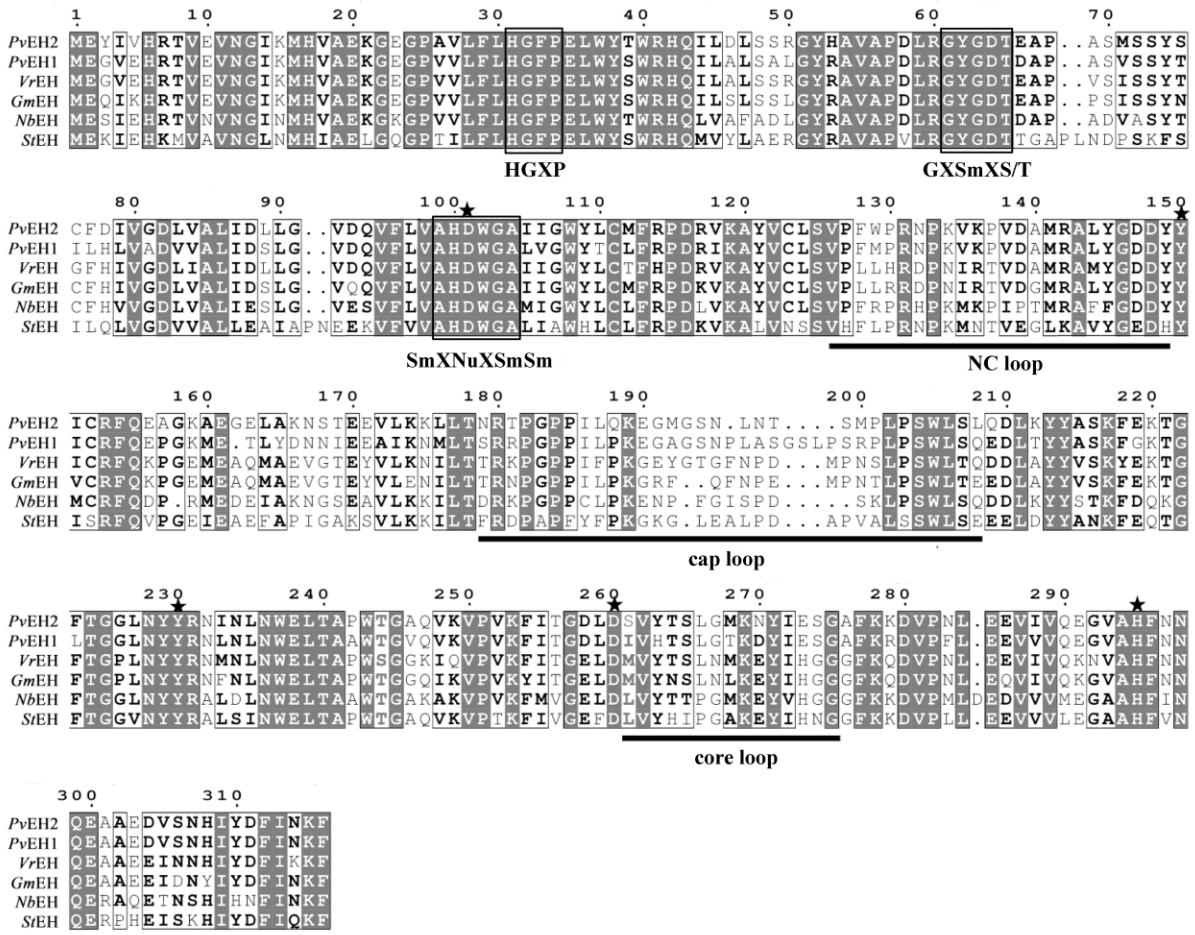


Figure 2

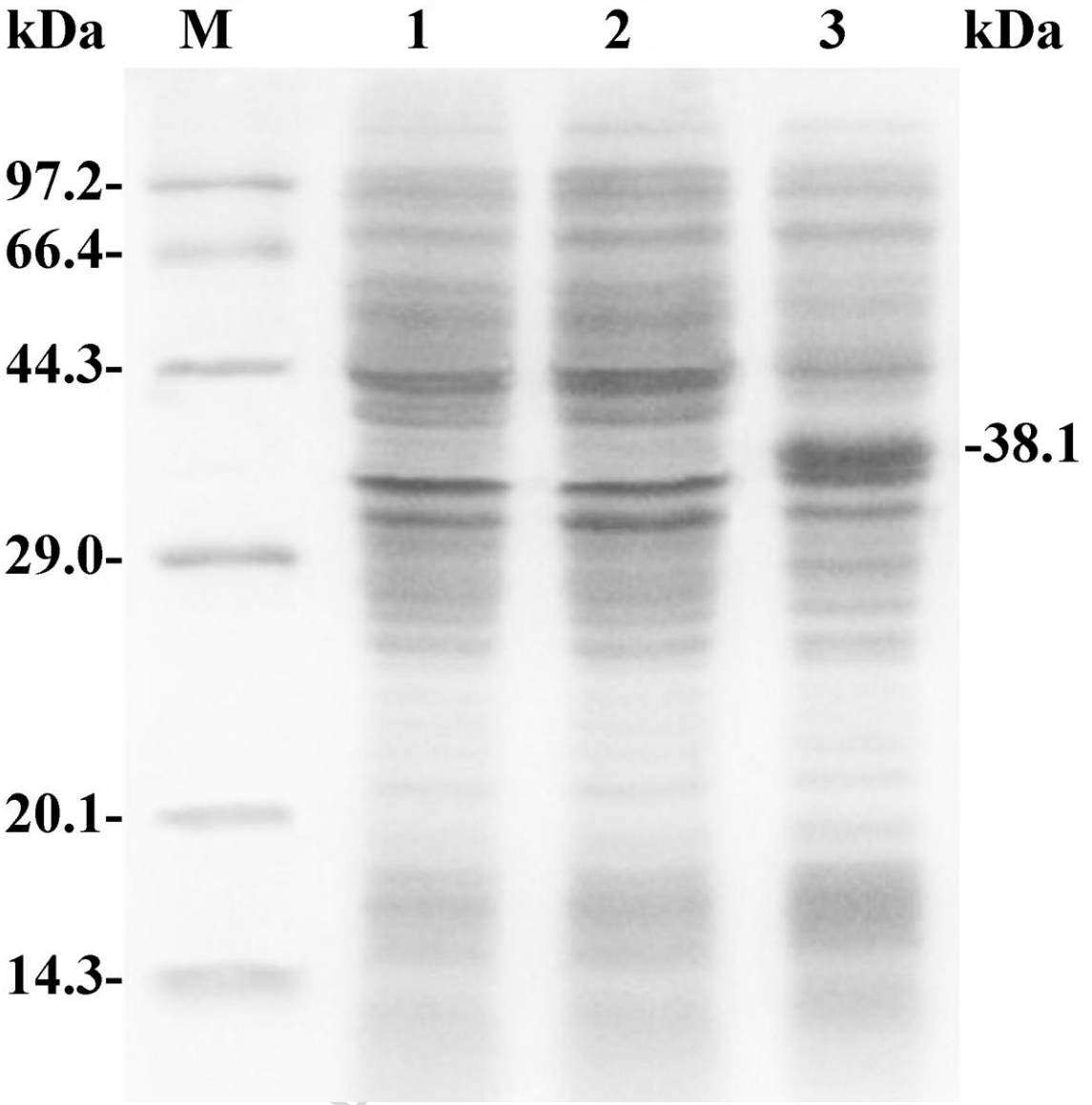


Figure 3

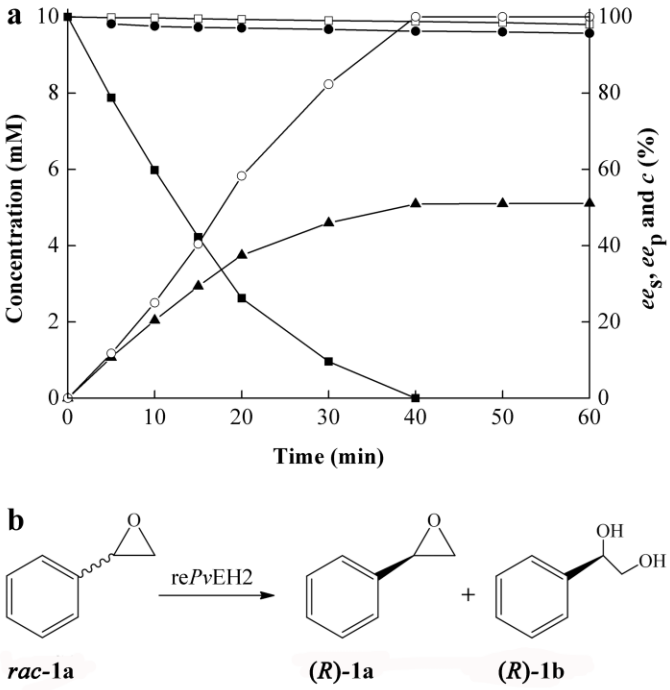
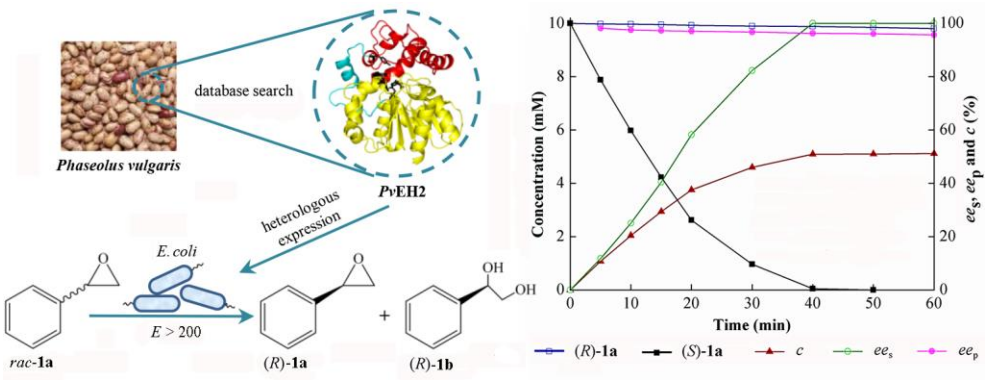


Figure 4



Graphical abstract

Highlights

- The newly discovered re*Pv*EH2 shows a very high *E* value (> 200) towards racemic styrene oxide (SO).
- The α_S and β_R of re*Pv*EH2 towards (*S*)- and (*R*)-SO are 99.1 and 69.8%, respectively.
- (*R*)-SO with > 99.5% ee_s and (*R*)-phenyl-1,2-ethanediol with 96.2% ee_p were co-produced.
- Besides SO, re*Pv*EH2 also exhibits good catalytic properties towards other epoxides.