ORGANIC LETTERS

2006
Vol. 8, No. 8
1737−1740

Enantioconvergent Hydrolysis of Styrene Epoxides by Newly Discovered Epoxide Hydrolases in Mung Bean

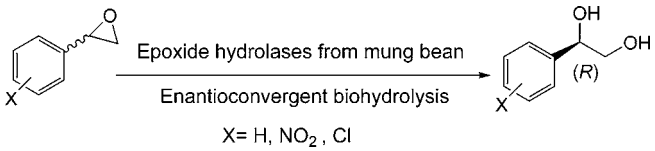
Wei Xu,[†] Jian-He Xu,*[,†] Jiang Pan,[†] Qing Gu,[‡] and Xin-Yan Wu[‡]

Laboratory of Biocatalysis and Bioprocessing, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China, and Institute of Fine Chemicals, East China University of Science and Technology, Shanghai 200237, China

jianhexu@ecust.edu.cn

Received February 16, 2006

ABSTRACT



Two novel epoxide hydrolases were discovered in mung bean (*Phaseolus radiatus L.*) for the first time, either of which can catalyze enantioconvergent hydrolysis of styrene epoxides. Their regioselectivity coefficients are more than 90% for the *p*-nitrostyrene oxide. Furthermore, the crude mung bean powder was also shown to be a cheap and practical biocatalyst, allowing a one-step asymmetric synthesis of chiral (*R*)-diols from racemic epoxides, in up to >99% ee and 68.7% overall yield (after recrystallization).

Enantiopure epoxides and their corresponding vicinal diols are important chiral building blocks for the pharmaceutical industry. Of particular importance are styrene oxide derivatives, such as **8a**, **9a**, **10a**, and **12a** in Figure 1, which are the essential moieties for preparing antagonists of central and peripheral dopamine receptors and for several promising medicinal substances showing antiobesity and antidiabetic activities.[1]

Epoxide hydrolase (EH) is such a nature-given tool for asymmetric hydrolysis of epoxides.[2] In recent decades, much attention has been paid to microbial epoxide hydrolases. The two pioneer teams in this field have been the Faber and Furstoss groups.[2] However, EHs from plant origins, discovered much earlier,[1] have had scarce further practical application in asymmetric catalysis until 2004 in work by the Furstoss group.[2]

The ideal way to obtain optically pure drug enantiomers from racemic precursors would be enantioconvergency. Enantioconvergency is one of the best strategies that allow overcoming the 50% maximum yield intrinsic to a classical resolution process.[3] It has been realized by chemoenzymatic processes or a combination of two enantiocomplementary enzymes, or even one enzyme as catalyst.[1c,4] In this context, a simple biological method was described to take one-step enantioconvergency into practice.

After extensive screening from edaphon and several kinds of plant for enantioselective epoxide hydrolases using **8a** as

[†] State Key Laboratory of Bioreactor Engineering.
[‡] Institute of Fine Chemicals.

(1) (a) Murmann, W.; Rumore, G.; Gamba, A. *Bull. Chim. Farm.* **1967**, *106*, 251−268. (b) Wierenga, W.; Evans, B. R.; Zurenko, G. E. *J. Med. Chem.* **1984**, *27*, 1212−1215. (c) Moreau, S. P.; Morisseau, C.; Baratti, J.; Zylber, J.; Archerlas, A.; Furstoss, R. *Tetrahedron* **1997**, *53*, 9707−9714. (d) Kazusaki, M.; Kawabata, H. *Chromatography* **2004**, *25*, 61−65.

(2) (a) Archer, I. V. J. *Tetrahedron* **1997**, *53*, 15617−15662. (b) Tang, Y. F.; Xu, J. H.; Ye, Q.; Schulze, B. *J. Mol. Catal. B: Enzym.* **2001**, *13*, 61−68. (c) Spelberg, J. H. L.; Rink, R.; Kellogg, R. M.; Janssen, D. B. *Tetrahedron: Asymmetry* **1998**, *9*, 459−466. (d) Yeates, C. A.; van Dyk, M. S.; Botes, A. L.; Breytenbach, J. C.; Krieg, H. M. *Biotechnol. Lett.* **2003**, *25*, 675−680. (e) Kim, H. S.; Lee, S. J.; Lee, E. J.; Hwang, J. W.; Park, S.; Kim, S. J.; Lee, E. Y. *J. Mol. Catal. B: Enzym.* **2005**, *37*, 30−35. (f). Liu, Z. Y.; Michel, J.; Wang, Z. S.; Witholt, B.; Li, Z. *Tetrahedron: Asymmetry* **2006**, *17*, 47−52.

(3) (a) Steinreiber, A.; Faber, K. *Curr. Opin. Biotechnol.* **2001**, *12*, 552−558. (b) Archelas, A.; Furstoss, R. *Curr. Opin. Chem. Biol.* **2001**, *5*, 112−119. (c) Steinreiber, A.; Mayer, S. F.; Saf, R.; Faber, K. *Tetrahedron: Asymmetry* **2001**, *12*, 1519−1528. (d) Orru, R. V. A.; Archelas, A.; Furstoss, R.; Faber, K. *Adv. Biochem. Eng. Biotechnol.* **1998**, *63*, 145−167.

10.1021/ol060407u CCC: $33.50
Published on Web 03/25/2006

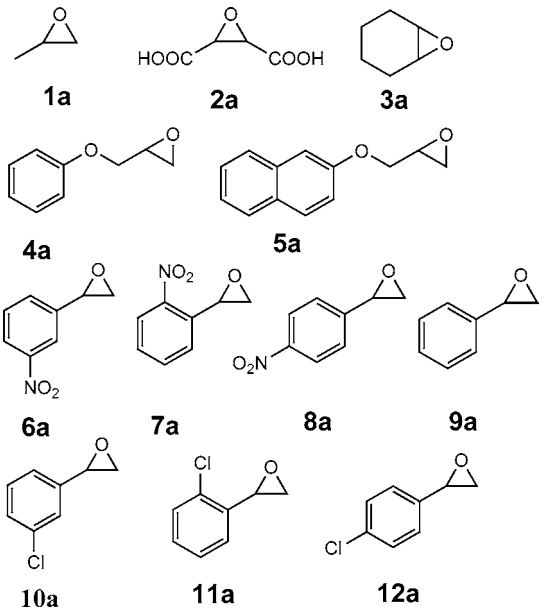


Figure 1. Epoxides tested as substrate.

a model substrate, mung bean was finally selected as a novel origin because it contains unique enzymes with the potential to catalyze enatioconvergent hydrolysis of styrene oxide derivatives. Mung bean, also referred to as green bean, is the seed of *Phaseolus radiatus L.* (an annual herb grown in the majority of areas in China). Owing to its extensive cultivation, it is very attractive to utilize this vegetable as a cheap and easily available source of EHs for synthetic purposes.

A series of interesting phenomena were observed in the subsequent studies. Unexpectedly, a preliminary purification of the enzyme shed light on the presence of two epoxide hydrolases, EH A and EH B, in mung bean (Figure 2)[5] Either of them catalyzed the enantioconvergent biohydrolysis of epoxide **8a**. Even more surprisingly, their enantiospecificity for **8a** are complementary, as evident in chromatograms A and B of Figure 2. EH A had the same enantiopreference as crude mung bean powder, while EH B had the opposite enantiopreference. The mechanisms for enantioconvergent biohydrolysis of racemic **8a** catalyzed by mung bean epoxide hydrolases (mbEHs) A and B were illustrated in Scheme 1. It is indicated that both mbEHs A and B attack (*S*)-**8a** (91.8% and 93.3%, respectively) mainly at the benzylic carbon (C_α) via an S_N2 mechanism. Thus an inversion of configuration occurs, producing (*R*)-**8b**. However, as for (*R*)-**8a**, since the terminal carbon (C_β) is primarily (93.0% and 90.2%, respectively) attacked by mbEHs A and B, the configuration is retained, affording also (*R*)-**8b**. Overall, both of the enzymatic processes lead to (*R*)-**8b** with a 100% theoretical yield. The only difference between mbEHs A and B is the opposite preference to enantiomers of substrate **8a**.

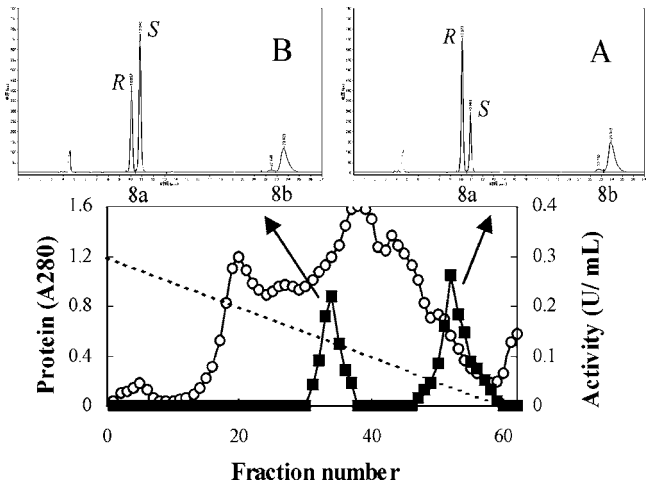


Figure 2. Elution profile of mbEH by hydrophobic chromatography on a Toyopearl butyl-650 M column. Parts A and B are the representative HPLC chromatograms on Chiralpak AD for the samples of **8a** hydrolysis catalyzed by mbEH fractions A and B. Symbols: ○, protein absorption at 280 nm (A_{280}); ■, EH activity on substrate **8a** (1 U=1 μmol/min); - - -, relative concentration of $(NH_4)_2SO_4$.

To demonstrate the occurrence of two EHs rather than two conformations of one enzyme, experiments were carefully designed by mixing the two fractions of partially purified enzyme in different proportions to perform the biohydrolysis of **8a**. As a result, the content of two enantiomers of residual **8a** varied linearly with the proportion of the two enzymes.

These results prompted us to characterize the two EHs. From the characteristic parameters listed in Table 1, it is obvious that, as far as the activity is concerned, the two partially purified enzymes are similar to each other, except for the Michaelis−Menten constant K_m.[9] The fact that K_m

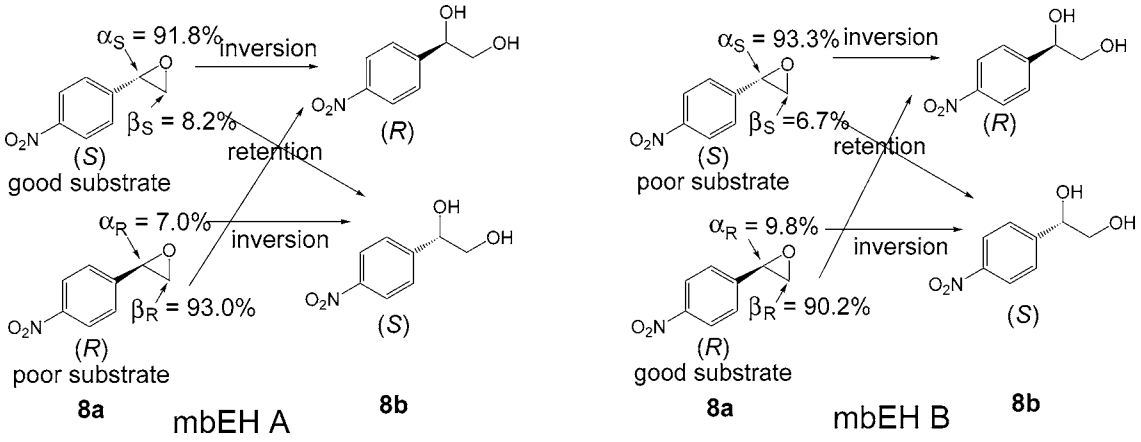
Table 1. Apparent Parameters of the Partially Purified EHs A and B for the Hydrolysis of Epoxide **8a**[a]

EH	optimal pH	optimal T (°C)	V_{max} (nmol·min^{-1} mg protein^{-1})	K_m (mM)
A	6.5	40	25.90 ± 1.02	0.26 ± 0.02
B	6.5	40	29.30 ± 0.11	1.48 ± 0.18

[a] Experiments were performed in triplicate, and the standard deviations are given.

(4) Croteau, R.; Kolattukudy, P. E. *Arch. Biochem. Biophys.* **1975**, *170*, 73−81.
(5) Monterde, M. I.; Lombard, M.; Archelas, A.; Cronin, A.; Arandb, M.; Furstoss, R. *Tetrahedron: Asymmetry* **2004**, *15*, 2801−2805.
(6) (a) Orru, R. V. A.; Faber, K. *Curr. Opin. Chem. Biol.* **1999**, *3*, 16−21. (b) Wolfgang, K.; Martin, M.; Peter, P.; Faber, K. *Tetrahedron Lett.* **1996**, *37*, 8379−8382. (c) Vries, E. J.; Janssen, D. B. *Curr. Opin. Biotechnol.* **2003**, *14*, 414−420.
(7) (a) Blée, E.; Schuber, F. *J. Biol. Chem.* **1992**, *267*, 11881−11887. (b) Faber, K.; Mischitz, M.; Kroutil, W. *Acta Chem. Scand.* **1996**, *50*, 249−258. (c) Moreau, S. P.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1993**, *58*, 8: 5533−5536.
(8) A similar discovery of EH from fungus *Beauveria bassiana.* was reported; see: Moussou, P.; Archerlas, A.; Furstoss, R.; Baratti, J. C. *Enzyme Microb. Technol.* **2000**, *26*, 414−420.

Scheme 1



of EH A is smaller than that of EH B may serve as a sound explanation for why the crude mung bean presents the same enantiopreference as EH A, despite the coexistence of EH B.

To explore the application of the mbEHs, epoxide compounds **1a**−**5a** (Figure 1) were selected as substrates, and the milled mung bean was used directly as biocatalyst for convenience. Unfortunately, no significant enzymatic hydrolysis could be detected for **1a** and **2a**, and moderate to low enantioselectivity was obtained for **4a** and **5a**, giving the enantiomeric ratios (*E* values)[10] of 4.57 and 3.34. As for the meso epoxide **3a**,[11] milled mung bean hardly showed any enantioselectivity, while EHs A and B showed *E* values[12] of 3.8 and 1.7, respectively.

Experiments were carried out in triplicate, and the standard deviations are given. The spontaneous hydrolysis of epoxides has been subtracted.

To investigate the effect of substituents on the enantioconvergency, **6a**−**12a** were employed as substrates. In this case, *E* values are no longer applicable for the evaluation of stereoselectivity; instead, regioselectivity coefficients α_S and α_R were calculated using eq 1[14d] (Table 2). The regioselectivity coefficient has been used extensively to interpret the enantioconvergency.[14]

$$ee_p = \alpha_S - \alpha_R + \frac{ee_s(1 - \alpha_S - \alpha_R)(1 - c)}{c} \qquad (1)$$

Conclusions can be drawn from Table 2. First, the ortho-substituted styrene oxides **7a** and **11a** are poor substrates for mbEHs, indicating that the ortho substitutents have significant influence on the reactivity of mbEHs. On the other hand, as a general feature, both EHs preferentially hydrolyze the (*S*) antipode of the meta- and para-substituted styrene oxide derivatives to afford the (*R*) diols in an enantioconvergent manner. However, the two EHs exhibit a complementary enantioselectivity, which is an exception, only for **8a**. Compound **8a** is also the optimum substrate with excellent regioselectivity; both of the two mbEHs' regioselectivity coefficients are more than 90%. It is noteworthy that during the biohydrolysis of racemic **6a** the enantioselectivity reversed distinctly, which is peculiar in asymmetric

(9) Michaelis, L.; Menten, M.; Invertinwirkung, D. K. *Biochem. Z.* **1913**, *49*, 333−369.

Table 2. Regioselectivity of mbEHs A and B for Biohydrolysis of Racemic Epoxides **6a**−**12a**[a]

	mbEH A				mbEH B			
			abs config[b]				abs config[b]	
substrate	α_S[a] (%)	α_R[a] (%)	residual epoxide	diol	α_S[a] (%)	α_R[a] (%)	residual epoxide	diol
6a	82 ± 2	7 ± 2	*S*→*R*[c]	*R*	52 ± 0	0	*S*→*R*[c]	*R*
8a	92 ± 0.1	7.0 ± 1	*R*	*R*	93 ± 6	10 ± 1	*S*	*R*
9a	83 ± 1	32 ± 2	*R*	*R*	84 ± 1	31.4 ± 2	*R*	*R*
10a	79 ± 2	0	*R*	*R*	74 ± 3	11 ± 4	*R*	*R*
12a	nt[d]	nt[d]	nt[d]	*R*	nt[d]	nt[d]	nt[d]	*R*
7a/11a	nd[e]							*R*

[a] α_S and α_R are regioselectivity coefficients related to the attacks at the C_α carbon atom of the *S* and *R* enantiomers, respectively. α_S and α_R were calculated from three sets of data (conversion, ee_s, ee_p) at seven different time points during the biohydrolysis of racemic epoxides using the Nonlinear Curve Fitting function of Origin software according to eq 1. [b] The absolute configurations of **6a**, **8a**, **9a**, **10a**, **6b**, **8b**, **9b**, **10b**, and **12b** were established by comparison with the results of *Aspergillus niger* EH-catalyzed epoxide biohydrolysis.[33] [c] The enantioselectivity reversed during the biohydrolysis process. [d] Not tested, since ee_s could not be measured under the present conditions. [e] No product was detected.

biohydrolysis. The regioselectivity level of mbEHs is comparable to that of a recombinant *Solanum tuberosum* EH (another EH known for enantioconvergent potential for **9a**−**12a**).[5]

Although several EHs have been purified to homogeneity,[15] the preparative-scale reactions have to be operated with whole cells as the biocatalysts.[16] For practical consideration, we carried out a preparative-scale biohydrolysis using the crude powder of mung bean as biocatalyst and several commercially significant epoxides **8a**, **10a**, and **12a** as substrates, under the above conditions without any optimization. The results on total conversion are listed in Table 3. Taking **8a** for example, the biohydrolysis of 600 mg of substrate by 40 g of crude powder of mung bean afforeded 501 mg of (*R*)-**8b** in 82.4% ee and 83.5% yield after 48 h. The enantiopurity of this diol could be easily enhanced up to >99% ee by recrystallization with $CHCl_3$ with an overall yield of 68.7%, which indicates a promising way to one-step synthesis of chiral diols from racemic epoxides.

To the best of our knowledge, this is the first report of the EH activities in mung bean. The two EHs behaved quite differently from EHs reported previously.[17] The enantioconvergent potential makes them attractive tools for organic sythesis. Moreover, the crude powder of mung bean was shown to be an economical and practical biocatalyst. Further studies are in progress in our laboratory to optimize the reaction conditions, with the intention enhancing the utility of mbEHs for preparative-scale application.

Table 3. Preparative-Scale Biohydrolysis Using Crude Powder of Mung Bean as Biocatalyst

	biohydrolysis		after recrystallization	
substrate	ee_p (%) (total conversion)	yield (%)	ee_p (%)	overall yield (%)
8a	82.4	83.5	>99[a]	68.7
10a	62.3	77.4	NA[b]	NA[b]
12a	60.7	88.4	74.5[c]	69.2

[a] Recrystallization solvent: $CHCl_3$. [b] Not available. **10b** exsisted in an oily state, which could not be recrystallized. [c] Recrystallization solvent: petroleum ether/ethyl actate mixture.

Acknowledgment. This research was financially supported by the National Natural Science Foundation of China (Nos. 20272013, 20506037, and 203900506), Ministry of Education (No. 20050251011), and Ministry of Science and Technology, P.R. China (No. 2003CB716008). Cordial thanks are also given to Dr. Zhi Li at the Department of Chemical & Biomolecular Engineering, National University of Singapore, for his constructive discussion; to Prof. Zu-Yi Li, at Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, for his kind donation of (±)-**6b** as a reference compound; and to Profs. Xi-Kang Yan and Yu Wang at ECUST for their valuable suggestions in the manuscript preparation.

Supporting Information Available: Detailed description of experimental procedures and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL060407U

(10) *E* values were calculated using Sih's equation: Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

(11) Chang, D. L.; Wang, Z. S.; Heringa, M. F.; Wirthner, R.; Witholt, B.; Li, Z. *Chem. Commun.* **2003**, 960−961.

(12) The *E* values were calculated as $E = (1 + ee_p)/(1 - ee_p)$ according to: Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559−571.

(13) Jin, H.; Li, Z. Y.; Dong X. W. *Org. Biomol. Chem.* **2004**, *2*, 408−414.

(14) (a) Moussou, P.; Archelas, A.; Baratti, J, Furstoss, R. *J. Mol. Catal. B: Enzym.* **1998**, *5*, 213-217. (b) Moussou, P.; Archerlas, A.; Furstoss, R.; Baratti, J. C. *Enzyme Microb. Technol.* **2000**, *26*, 414−420. (c) Faber, K.; Kroutil, W. *Tetrahedron: Asymmetry* **2002**, *13*, 377−382. (d) Moussou, P.; Archelas, A.; Baratti, J.; Furstoss, R. *Tetrahedron: Asymmetry* **1998**, *9*, 1539−1547.

(15) (a) Blee, E.; Schuber, F. *Biochem. J.* **1992**, *282*, 711−714. (b) Nakamura, T.; Nagasawa, T.; Yu, F.; Watanabe, I.; Yamada, H. *Appl. Environ. Microbiol.* **1994**, *60*, 4630−4633. (c) Morisseau, C.; Archelas, A.; Guitton, C.; Faucher, D.; Furstoss, R.; Baratti, J. C. *Eur. J. Biochem.* **1999**, *263*, 386-395.

(16) (a) Pan, J.; Xu, J. H. *Enzyme Microb. Technol.* **2003**, *33*, 527−533. (b) Xu, Y.; Xu, J. H.; Pan, J.; Zhao, L.; Zhang, S. L. *J. Mol. Catal. B: Enzym.* **2004**, *27*, 155−159. (c) Xu, Y.; Xu, J. H.; Pan, J.; Tang, Y. F. *Biotechnol. Lett.* **2004**, *26*, 1217−1221. (d) Gong, P. F.; Xu, J. H. *Enzyme Microb. Technol.* **2005**, *3*, 252−257.

(17) (a) Smit, M. S. *Trends Biotechnol.* **2004**, *22*, 123−129. (b) Archer, I. V. J. *Tetrahedron* **1997**, *53*, 15617−15662. (c) Weijers, C. A. G. M.; de Bont, J. A. M. *J. Mol. Catal. B: Enzym.* **1999**, *6*, 199−214.