Biocatalytic conversion of epoxides

de Vries, Erik; Janssen, DB

Published in:
Current Opinion in Biotechnology

DOI:
10.1016/S0958-1669(03)00102-2

Biocatalytic conversion of epoxides

Erik J de Vries and Dick B Janssen*

Epoxides are attractive intermediates for producing chiral compounds. Important biocatalytic reactions involving epoxides include epoxide hydrolase mediated kinetic resolution, leading to the formation of diols and enantiopure remaining substrates, and enantioconvergent enzymatic hydrolysis, which gives high yields of a single enantiomer from racemic mixtures. Epoxides can also be converted by non-hydrolytic enantioselective ring opening, using alternative anionic nucleophiles; these reactions can be catalysed by haloalcohol dehalogenases. The differences in scope of these enzymatic conversions is related to their different catalytic mechanisms, which involve, respectively, covalent catalysis with an aspartate carboxylate as the nucleophile and non-covalent catalysis with a tyrosine that acts as a general acid-base. The emerging new possibilities for enantioselective biocatalytic conversion of epoxides suggests that their importance in green chemistry will grow.

Addresses
Department of Biochemistry, Groningen Biomolecular Sciences & Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands
*e-mail: d.b.janssen@chem.rug.nl

Current Opinion in Biotechnology 2003, **14**:414–420

This review comes from a themed issue on
Protein technologies and commercial enzymes
Edited by Gjalt Huisman and Stephen Sligar

0958-1669/$ – see front matter

DOI 10.1016/S0958-1669(03)00102-2

Abbreviations
ee enantiomeric excess
GST glutathione *S*-transferase

Introduction

Because of the intrinsic reactivity of the epoxide ring, epoxides are valuable intermediates in the production of high added-value chemicals like pharmaceuticals. Racemic mixtures of epoxides can be used to prepare enantiopure epoxides by kinetic resolution and a range of chemical [1] and biocatalytic [2] procedures are available for this purpose. Epoxides are attractive for further derivatization because they readily react with halides, carbon, nitrogen, oxygen or sulfur nucleophiles. For example, the conversion of glycidol derivatives with amines is a useful step during the preparation of various β-blockers. It has been demonstrated that at least some of these coupling reactions have an enzyme-catalysed equivalent. Biocatalytic epoxide conversion can proceed by hydrolysis, conjugation to thiol cofactors, and through nucleophilic ring opening. The most important enzyme classes are shown in Table 1.

Several interesting concepts that have been developed over the past years for other biocatalysts might also be applicable for the enzymatic conversion of epoxides. One attractive possibility would be to tailor the nucleophile in epoxide ring-opening reactions. With lipases and esterases, for instance, this can be used (in organic solvents) to prepare amides by adding an amine to the reaction mixture [3]. Similarly, glycosidases can couple a sugar moiety to an alcohol [4,5] and penicillin acylases can be used for the resolution of amines through acylation [6]. In some cases, protein engineering by mutagenesis or directed evolution has resulted in dramatically altered enzymatic activities, yielding enzymes that form different reaction products compared with wild-type enzymes [7,8]. It is desirable to expand the current biosynthetic toolbox for epoxide conversion by finding new enzymes or by applying the above-mentioned concepts to broaden the scope and performance of existing epoxide-converting biocatalysts. Here we present recent advances in the biocatalytic conversion of epoxides. We describe the mechanisms of epoxide-converting enzymes and discuss the use of different nucleophiles in enzymatic ring-opening reactions.

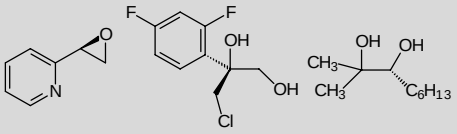
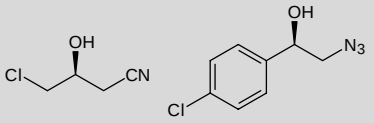
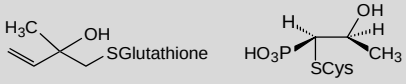
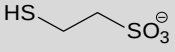
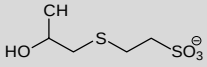
Epoxide ring opening with water using epoxide hydrolase

The hydrolysis of epoxides to the corresponding diols with epoxide hydrolases has been well described [9••, 10,11]. It is known that epoxides can be opened by direct attack of the nucleophile on the epoxide ring or via an intermediate in which there is a covalent link between the enzyme and the substrate (covalent catalysis). Most epoxide hydrolases belong to the α/β-hydrolase fold family of enzymes, which also encompasses many lipases, esterases and dehalogenases. The mechanism of epoxide hydrolase from *Agrobacterium radiobacter* AD1 is shown in Figure 1a [12]. The *Aspergillus niger* enzyme has a similar structure and mechanism [13]. The enzymes possess a catalytic triad, of which the nucleophilic aspartate carries out an attack on the carbon atom of the epoxide ring, displacing the oxygen and producing a covalent intermediate. Hydrolysis of this intermediate by water is facilitated by a histidine residue. This regenerates the enzyme and gives the product as a diol of which one of the oxygen atoms originates from the enzyme.

It is assumed that in most cases the proton transfer to the oxygen of the substrate is concerted with the attack of the aspartate or that it immediately follows the ring opening.

Table 1

Important enzyme-catalysed conversions of epoxides in microorganisms.

Enzyme	Accepted nucleophile(s)	Examples of product(s)
Epoxide hydrolase	H_2O	
Haloalcohol dehalogenase	$Cl^{\ominus}$, $Br^{\ominus}$, $I^{\ominus}$, $CN^{\ominus}$, $N_3^{\ominus}$, $NO_2^{\ominus}$	
Glutathione-*S*-transferase	GSH, Cys	
CoM-transferase		

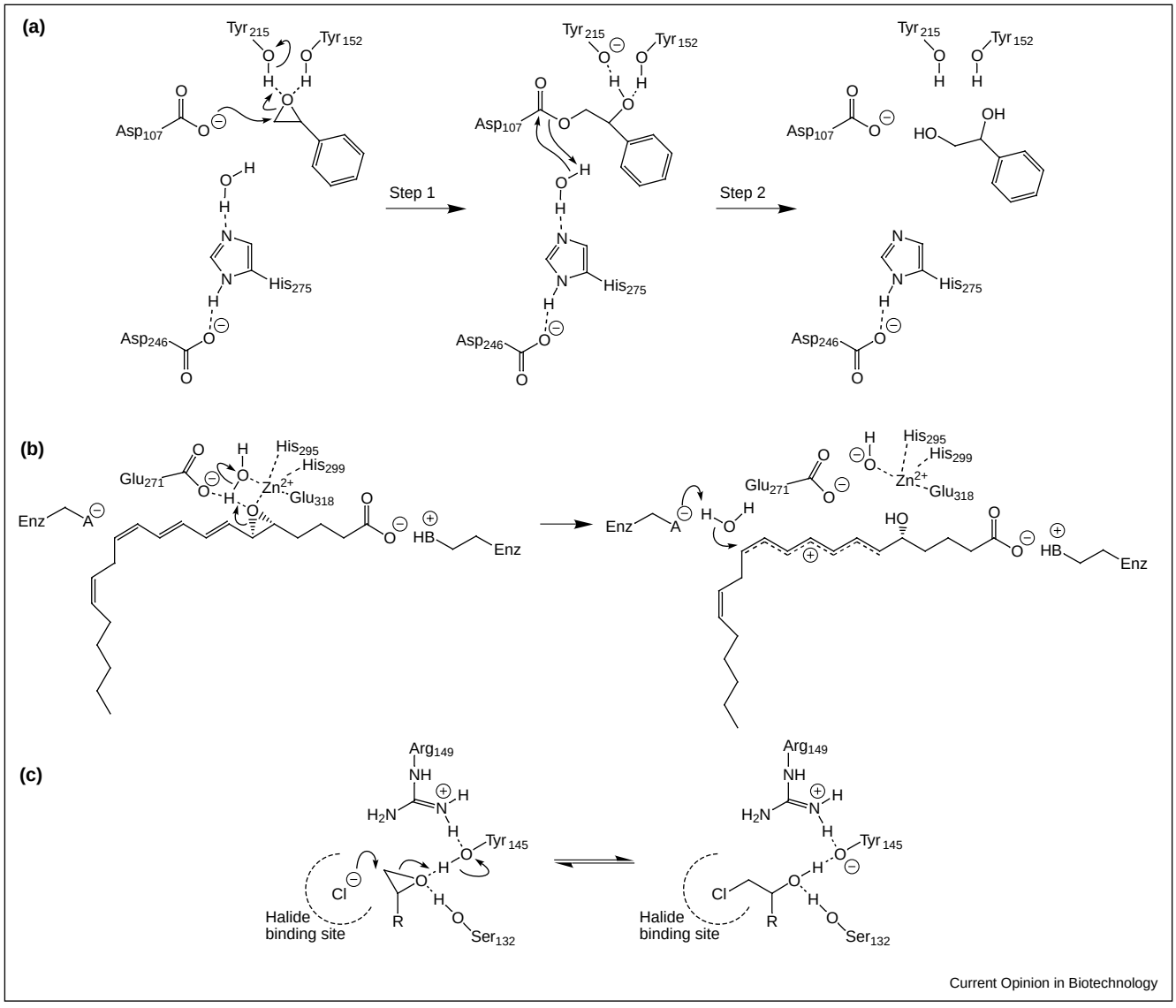
If electron withdrawing or donating effects of the *para*-substituent on the aromatic ring affect the rate-limiting step, this should give a clear correlation in a so-called Hammett plot where the relative reaction rate is plotted versus the Hammett constant σ_p, which represents the electron-withdrawing effect of the ring substituent. However, Hammett plots of the hydrolysis of *para*-substituted styrene oxides by some epoxide hydrolases did not show this correlation, which was regarded as evidence for the involvement of a general acid in catalysis, indicating epoxide oxygen protonation as a distinct first step [14]. Proof for this mechanism was also obtained with a crude rabbit liver microsome preparation [15], where protonation by a general acid seemed to be the first step during the conversion of norbornene-2,3-epoxide, as there was a shift in the position of the hydroxyl in the product which could only be explained by the transient formation of a carbocation that has sufficient lifetime to allow a rearrangement to occur. Another explanation for the lack of a correlation in a Hammett plot might be that the hydrolysis of the covalent intermediate rather than its formation is the rate-limiting step, which is the case with at least some substrates for the *A. radiobacter* enzyme [16].

Developments in the field of molecular biology and advances in high-throughput screening methods [17••] continue to increase the number and amounts of available epoxide hydrolase and also make it possible to improve their properties. For screening of epoxide hydrolases with improved enantioselectivity, the pseudo-enantiomers (*S*)-glycidyl phenyl ether and (*R*)-D-5-glycidyl phenyl ether were synthesized and used to quantify the enantiomeric excess (ee) value by multichannel mass spectrometry [18]. However, mutants with improved enantioselectivity have, thus far, only been obtained by structure-based site-directed mutagenesis [19].

By screening a range of enzymes and mutants, the ones that are most suited for the conversion of a specific substrate can be selected [20•]. This has led, for instance, to the availability of epoxide hydrolases for pyridyloxiranes, which are notoriously difficult to prepare in an optically pure form by chemical methods. Using epoxide hydrolase from *A. niger* [21] or a mutated *A. radiobacter* enzyme [19], resolution of 2-, 3-, and 4-pyridyloxirane was obtained on a scale of up to 36 g/l. By selecting the correct enzymes, enantioconvergent hydrolysis can be obtained, which is based on different regioselectivities for the different enantiomers [22], and allows isolation of the diols in more than 50% yield. The strength of this concept is demonstrated in the hydrolysis of aliphatic trisubstituted epoxides: with *Streptomyces lavendulae* epoxide hydrolase the diol was obtained in 60% yield with an ee of 97% and a *Rhodococcus ruber* enzyme gave 85% yield with an ee of 85% [23••]. A further example concerns the production of *para*-chlorostyrene epoxide [24]. In this case, the (*S*)-enantiomer of the racemic mixture was hydrolyzed by *Solanum tuberosum* EH which forms the (*R*)-diol by attack on the benzylic position, inverting the stereochemistry. In a consecutive step with *A. niger* EH the unreacted (*R*)-enantiomer that was left behind reacts on the least substituted carbon atom to also form the (*R*)-diol. Using this strategy, the (*R*)-diol could be isolated in 93% yield with an ee of 96%.

The use of epoxides in biocatalytic processes causes some challenges related to the chemical properties of epoxides. Several substrates are poorly soluble and the reactivity of the epoxide ring can cause chemical hydrolysis. As a remedy, the substrate can be added as a solid or in a liquid phase. Another method uses octane as a second phase, which dissolves the epoxide. This approach

Figure 1



Reaction mechanisms of epoxide-converting enzymes. **(a)** Ring opening of styrene oxide by epoxide hydrolases from *A. radiobacter*. Step 1, nucleophilic attack of Asp107 yielding a covalent intermediate. Step 2, hydrolysis of the covalent intermediate by water, assisted by His275. **(b)** Non-covalent catalytic mechanism of leukotriene A4 epoxide hydrolase. Ring opening is facilitated by a zinc atom. **(c)** Reversible ring opening of an epoxide by *A. radiobacter* haloalcohol dehalogenase. The arginine–tyrosine pair is involved in leaving group protonation.

allowed the enantioselective hydrolysis of styrene oxide on a 39 g/l scale with good yields of the remaining (*S*)-enantiomer [25]. Immobilization techniques can enhance the enzyme stability, change the catalytic properties, and facilitate the recycling of the biocatalyst. A flow-through membrane reactor can be used to retain enzyme and cosolvents to improve substrate solubility [26]. From the low ee values that were obtained, and the observation that enzyme inactivation occurred if the cosolvent (ethanol, acetonitrile) concentration was above 20%, it can be concluded that reactor aspects need to be improved further. Imprinting the enzyme with a substrate in the immobilization process can influence the enantioselectivity. *Rhodotorula glutinis* EH was imprinted with (*S*)-1,2-epoxyoctane before immobilization and it was found that the enantiospecificity in the hydrolysis of the racemic mixture of this substrate changed from (*R*)-specific to (*S*)-specific, albeit with low enantioselectivity, changing from E = 1.3 (*R*) to 1.8 (*S*) [27•]. The E-value allows for the easy comparison of enantioselective processes, and is defined as the ratio of the k_{cat}/K_m values for the enantiomers.

Epoxide ring opening with other nucleophiles

It would be attractive to be able to use nucleophiles other than water, for example, in the synthetic concepts mentioned in the introduction and in enantioselective kinetic resolutions. To our knowledge, no examples of using other nucleophilic agents with epoxide hydrolases have been reported. This is probably related to the mechanism of the αβ-hydrolase fold enzymes. For instance, replacing water in step 2 (Figure 1a) with ammonia would, after one turnover, change Asp192 into Asn192, which is not a suitable nucleophile for catalysis. An inactive Asp→Asn mutant reverts back to wild type by slow hydrolysis ($t_{1/2} = 9.3$ days) of the asparagine to aspartate [28]. This same turnover-related inactivation by ammonia is found in the fluoroacetate dehalogenase, which also uses a nucleophilic aspartate [29]. With this class of epoxide hydrolase, the covalent intermediate is always attacked at the carbonyl functionality of the enzyme and, unless the mechanism of breaking the covalent intermediate changes to attack on the substrate carbon atom, it is only possible to prepare vicinal diols.

There are epoxide hydrolases that act through a different mechanism. In the fungus *Fusarium solani pisi cis*-hydration of the epoxide occurs [30]. Based on inhibition studies, a cysteine residue was postulated to attack the epoxide ring, with inversion, to form a thioether intermediate. Attack of water at the same carbon atom results in inversion of stereochemistry again. Alternatively, the concept of covalent catalysis may be abandoned completely. In ring opening through non-covalent catalysis, the epoxide ring will be activated by interacting with certain groups of the enzyme, and ring opening will rely on the reactivity of an activated nucleophile. The mechanism of leukotriene A4 hydrolase is well known (Figure 1b) [31]. The expected vicinal diol is not formed owing to the nature of the substrate, which allows a shift of the carbocation intermediate. Other probable candidates for non-covalent catalysis are limonene epoxide hydrolase from *Rhodococcus erythropolis* [32] (see also Update) and cholesterol epoxide hydrolase [33]. It is conceivable that with such different enzymes alternative nucleophiles might be used.

Conversion of epoxides by haloalcohol dehalogenases

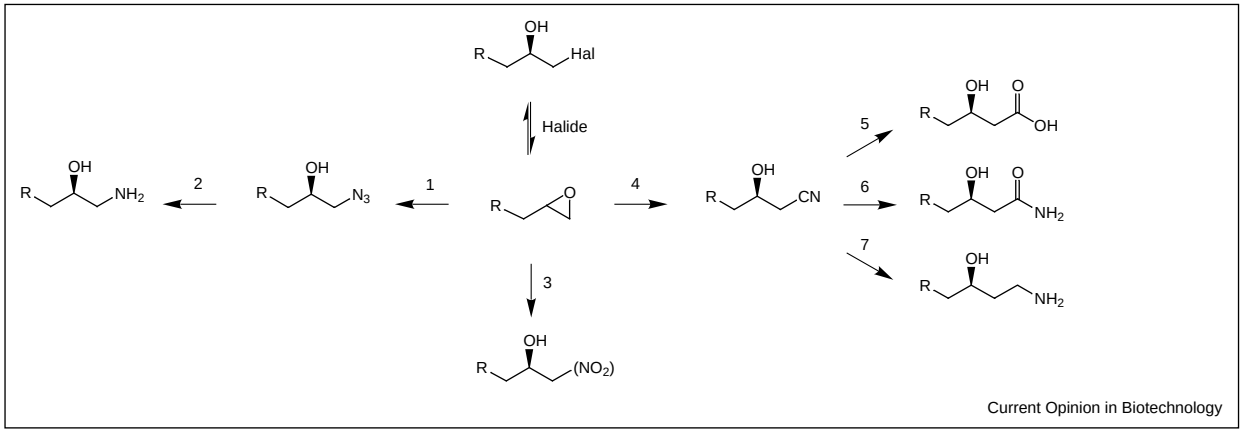
Haloalcohol dehalogenases (also called halohydrin dehalogenases or hydrogen-halide lyases) catalyse the reversible dehalogenation of vicinal haloalcohols by an intramolecular nucleophilic displacement of a halogen to yield an epoxide and halide. These enzymes play a role in the biodegradation of some xenobiotic halogenated compounds. In Figure 1c, the proposed mechanism of the reversible ring opening is shown, which is based on the sequence similarity with the short-chain dehydrogenase/reductase (SDR) family of proteins [34•]. The process involves activation of the epoxide by hydrogen bonding to a tyrosine. As found in other dehalogenases [35], the nucleophile is expected to bind in a halide-binding pocket.

The use of haloalcohol dehalogenases for kinetic resolution of haloalcohols by an enantioselective ring closure reaction is well documented [36] and this process is applied on an industrial scale for the production of optically pure epichlorohydrin. The fact that a non-covalent pathway is used suggests that it is easier to apply for the reverse reaction. Until recently, only one example of the reverse reaction with the alternative nucleophile cyanide, resulting in β-hydroxynitriles, was described [37]. A screening of possible nucleophiles accepted by the haloalcohol dehalogenase HheC from *A. radiobacter* has been published [38••]. A range of ionic and non-ionic nucleophiles were tested for their ability to react with a chromogenic epoxide. Besides the nucleophiles Cl^-, Br^- and I^-, the ambident nucleophiles N_3^-, NO_2^- and CN^- were also accepted by the enzyme. No conversion was observed with non-ionic nucleophiles such as isopropylamine and ethanol. This specificity of the enzymes towards certain nucleophiles must be due to a selective interaction in the halide-binding site. The non-halide nucleophiles accepted by the haloalcohol dehalogenase are ambident anions with a (close to) linear shape. This suggests that the binding site of the nucleophile has the shape of a tunnel and that binding at the non-reacting end of the nucleophile is stabilized by groups that donate a hydrogen bond.

Amino alcohols are important molecules and can be prepared from the azido alcohol through reduction. The ring opening of *para*-substituted styrene oxides by azide using haloalcohol dehalogenase was studied in detail [39]. Catalysis occurred with high β-regioselectivity and enantioselectivity ($E > 200$) towards the (*R*)-epoxide, resulting in the remaining (*S*)-epoxides and the formed (*R*)-2-azido-1-phenylethanols in high optical purity. The reaction was irreversible, as azide is a very poor leaving group. The regioselectivity towards the β-carbon atom is remarkable as it is opposite to the observed regioselectivity in the non-enzyme catalysed chemical ring opening. A first attempt at scale-up in which the azide was slowly added and *para*-nitrostyrene oxide was present as a solid second phase (7.8 g/l) yielded (*S*)-*para*-nitrostyrene oxide in 46% yield and 98% ee and the (*R*)-2-azido alcohol in 47% yield and 97% ee.

The nature of the product formed by ring opening with nitrite is not yet known. However, on the basis of the proposed shape of the halide-binding site, the formation of a nitrite ester is more likely than the formation of a nitro-compound. Further investigations of this mechanism by crystallographic studies are currently under way [40]. Based on the outcome of this study, the halide-binding site might be modified through site-directed mutagenesis to accept other nucleophiles.

Figure 2



Conversions of epoxides by haloalcohol dehalogenase and their biocatalytic potential. Enzymatic azidolysis (step 1) followed by reduction (step 2) gives β-amino alcohols. The product of enzymatic ring opening with nitrite (step 3) is not clear, it gives either the nitrite ester (nucleophilic O-attack) or the nitro alcohol (nucleophilic N-attack). Ring opening with cyanide (step 4) yields a β-cyanohydrin, which can be partially hydrolyzed to the amide (step 6), completely hydrolyzed to the acid (step 5) or reduced to the γ-amino alcohol (step 7).

With the nucleophiles that are known to be accepted by haloalcohol dehalogenases, it is possible to prepare enantiomerically pure haloalcohols, β- and γ-amino alcohols, β-hydroxynitriles and, thus, β-hydroxy amides and acids (Figure 2).

Other epoxide-converting enzymes

Several other routes for the microbial conversion of epoxides have been described. Glutathione S-transferases (GSTs) are involved in the detoxification of compounds by their conjugation to the tripeptide glutathione (γ-Glu-Cys-Gly), followed by further metabolization. Two distinct mechanisms are found for the conjugation of epoxides with glutathione. The most common one relies on hydrogen bonding of the thiol group of glutathione to suitable groups on the enzyme, making it more nucleophilic. Alternatively, complexation of the epoxide ring of the substrate to a metal ion like Mn(II) makes it more electrophilic, facilitating attack of glutathione [41]. In general, GSTs have a large solvent-exposed active site and, thus, a broad specificity. Over the past few years more microbial enzymes have become available. Their role in epoxide metabolism is still unclear and it is not known if these enzymes can be applied in enantioselective biocatalysis. The regioselectivity of ring opening has been studied for the conjugation of isoprene epoxides, but whether the reactions proceed with enantioselectivity was not established rigorously [42,43]. FosA is a member of the second group of glutathione transferases, using a metal ion for substrate-epoxide ring activation. In the detoxification of fosfomycin by ring opening, glutathione can be replaced by cysteine [44–46].

Epoxides are common intermediates in the microbial degradation of alkenes. In these cases, epoxide conversion can proceed via carboxylation, which is catalysed by a four-component multi-enzyme system that couples epoxides with carbon dioxide to form β-keto carboxylic acids [47,48]. This reaction requires cofactor M (CoM, 2-mercapto-1-ethyl sulfonate) [49,50]. The system was found to enantioselectively degrade 2,3-epoxybutane [51], but not epoxypropane. Component 1 of the carboxylase system has been isolated and purified; it is a zinc-containing hexamer performing a nucleophilic ring opening with CoM. Analysis of the isolated intermediate showed that the reaction occurs selectively at the least hindered carbon atom. Incubations of the racemate and the separate enantiomers revealed only a slight enantiopreference: (*R*)-propylene oxide reacted twice as fast as (*S*)-propylene oxide. Other thiols, such as mercaptoethanol, cysteine, glutathione or 3-mercapto-1-propane sulfonate were not active as a nucleophile [52].

Conclusions

The use of new methods for screening and selection of enzymes as well as the exploitation of unusual microbial sources has rapidly expanded the biocatalytic scope of epoxide-converting enzymes. The diversity of available enantioselective epoxide hydrolases has increased. New reactions have been found for haloalcohol dehalogenases, including enantioselective enzymatic azidolysis. The range of substrates for which attractive applications have been demonstrated is increasing. This justifies the conclusion that the importance of epoxides in green chemistry routes for the enantioselective preparation of fine chemicals will quickly grow.

Update

Recently, a protein crystallographic study showed that indeed limonene epoxide hydrolase has a novel fold and

acts through a one-step mechanism involving the protonation of the epoxide ring by aspartate 101 with concomitant attack of a water molecule on a carbon atom of the epoxide ring [53]. A study into the pre-steady state and steady state kinetics of the ring closure of haloalcohols by the haloalcohol dehalogenase of *A. radiobacter* AD1 led to a better understanding of the enantioselectivity of this enzyme [54].

Acknowledgements

Part of our work on epoxide hydrolases was funded by the EU (project number QLK3-CT-2000-00426, acronym: EPOX) and by the Netherlands Foundation of Chemical Research (CW) with financial support by the Netherlands Foundation for Scientific Research (NWO).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jacobsen EN: **Asymmetric catalysis of epoxide ring-opening reactions**. *Acc Chem Res* 2000, **33**:421-431.

2. Archelas A, Furstoss R: **Synthesis of enantiopure epoxides through biocatalytic approaches**. *Annu Rev Microbiol* 1997, **51**:491-525.

3. Gotor V: **Lipases and (*R*)-oxynitrilases: useful tools in organic synthesis**. *J Biotechnol* 2002, **96**:35-42.

4. Remond C, Ferchichi M, Aubry N, Plantier-Royon R, Portella C, O'Donohue MJ: **Enzymatic synthesis of alkyl arabinofuranosides using a thermostable α-L-arabinofuranosidase**. *Tetrahedron Lett* 2002, **43**:9653-9655.

5. Zechel DL, Withers SG: **Dissection of nucleophilic and acid-base catalysis in glycosidases**. *Curr Opin Chem Biol* 2001, **5**:643-649.

6. van Langen LM, Oosthoek NHP, Guranda DT, van Rantwijk F, Svedas VK, Sheldon RA: **Penicillin acylase-catalyzed resolution of amines in aqueous organic solvents**. *Tetrahedron Asymmetry* 2000, **11**:4593-4600.

7. Wu TK, Griffin JH: **Conversion of a plant oxidosqualene-cycloartenol synthase to an oxidosqualene-lanosterol cyclase by random mutagenesis**. *Biochemistry* 2002, **41**:8238-8244.

8. Wymer N, Buchanan LV, Henderson D, Mehta N, Botting CH, Pocivavsek L, Fierke CA, Toone EJ, Naismith JH: **Directed evolution of a new catalytic site in 2-keto-3-deoxy-6-phosphogluconate aldolase from *Escherichia coli***. *Structure* 2001, **9**:1-9.

9. •• Steinreiber A, Faber K: **Microbial epoxide hydrolases for preparative biotransformations**. *Curr Opin Biotechnol* 2001, **12**:552-558.
This review gives a comprehensive overview of substrates that can be hydrolyzed by the different classes of epoxide hydrolase.

10. Armstrong RN, Cassidy CS: **New structural and chemical insight into the catalytic mechanism of epoxide hydrolases**. *Drug Metab Rev* 2000, **32**:327-338.

11. Archelas A, Furstoss R: **Synthetic applications of epoxide hydrolases**. *Curr Opin Chem Biol* 2001, **5**:112-119.

12. Nardini M, Ridder IS, Rozeboom HJ, Kalk KH, Rink R, Janssen DB, Dijkstra BW: **The X-ray structure of epoxide hydrolase from *Agrobacterium radiobacter* AD1. An enzyme to detoxify harmful epoxides**. *J Biol Chem* 1999, **274**:14579-14586.

13. Zou J, Hallberg BM, Bergfors T, Oesch F, Arand M, Mowbray SL, Jones TA: **Structure of *Aspergillus niger* epoxide hydrolase at 1.8 Å resolution: implications for the structure and function of the mammalian microsomal class of epoxide hydrolases**. *Structure* 2000, **8**:111-122.

14. Moussou P, Archelas A, Baratti J, Furstoss R: **Microbiological transformations. 38. Clues to the involvement of a general acid activation during hydrolysis of *para*-substituted styrene oxides by a soluble epoxide hydrolase from *Syncephalastrum racemosum***. *J Org Chem* 1998, **63**:3532-3537.

15. Chiappe C, De Rubertis A, Marioni F, Simonetti A: **The anomalous course of the microsomal transformation of the exo-2,3-epoxides of norbornene and norbornadiene. The possible involvement of a general acid activation during the enzymatic hydrolysis of these oxides**. *J Mol Cat B Enz* 2000, **10**:539-544.

16. Lutje Spelberg JH, Rink R, Archelas A, Furstoss R, Janssen DB: **Biocatalytic potential of the epoxide hydrolase from *Agrobacterium radiobacter* AD1 and a mutant with enhanced enantioselectivity**. *Adv Synth Catal* 2002, **344**:980-985.

17. •• Mateo C, Archelas A, Furstoss R: **A spectrophotometric assay for measuring and detecting an epoxide hydrolase activity**. *Anal Biochem* 2003, **314**:135-141.
This article describes the development of a generic spectrophotometric assay for the determination of epoxide hydrolase activity.

18. Schrader W, Eipper A, Pugh DJ, Reetz MT: **Second-generation MS-based high-throughput screening system for enantioselective catalysts and biocatalysts**. *Can J Chem* 2002, **80**:626-632.

19. Genzel Y, Archelas A, Lutje Spelberg JH, Janssen DB, Furstoss R: **Microbiological transformations. Part 48: Enantioselective biohydrolysis of 2-, 3- and 4-pyridyloxirane at high substrate concentration using the *Agrobacterium radiobacter* AD1 epoxide hydrolase and its Tyr215Phe mutant**. *Tetrahedron* 2001, **57**:2775-2779.

20. • Genzel Y, Archelas A, Broxterman QB, Schulze B, Furstoss R: **Microbiological transformations 50: selection of epoxide hydrolases for enzymatic resolution of 2-, 3- or 4-pyridyloxirane**. *J Mol Cat B Enz* 2002, **16**:217-222.
Together with [19] and [21], this article describes the work on the hydrolysis of pyridyl oxiranes: selection of enzymes, determination of enantioselectivities and scale-up issues.

21. Genzel Y, Archelas A, Broxterman QB, Schulze B, Furstoss R: **Microbiological transformations. 47. A step toward a green chemistry preparation of enantiopure (*S*)-2-, -3-, and -4-pyridyloxirane via an epoxide hydrolase catalyzed kinetic resolution**. *J Org Chem* 2001, **66**:538-543.

22. Faber K, Kroutil W: **Stereoselectivity in biocatalytic enantioconvergent reactions and a computer program for its determination**. *Tetrahedron Asymmetry* 2002, **13**:377-382.

23. •• Steinreiber A, Mayer SF, Saf R, Faber K: **Biocatalytic asymmetric and enantioconvergent hydrolysis of trisubstituted oxiranes**. *Tetrahedron Asymmetry* 2001, **12**:1519-1528.
This article describes a very good example of enantioconvergence in the hydrolysis of an aliphatic epoxide. The total yield of almost enantiomerically pure product well exceeds the 50% level.

24. Manoj KM, Archelas A, Baratti J, Furstoss R: **Microbiological transformations. Part 45: A green chemistry preparative scale synthesis of enantiopure building blocks of Eliprodil: elaboration of a high substrate concentration epoxide hydrolase-catalyzed hydrolytic kinetic resolution process**. *Tetrahedron* 2001, **57**:695-701.

25. Baldascini H, Ganzeveld KJ, Janssen DB, Beenackers AA: **Effect of mass transfer limitations on the enzymatic kinetic resolution of epoxides in a two-liquid-phase system**. *Biotechnol Bioeng* 2001, **73**:44-54.

26. Krieg HM, Botes AL, Smith MS, Breytenbach JC, Keizer K: **The enantioselective catalytic hydrolysis of racemic 1,2-epoxyoctane in a batch and a continuous process**. *J Mol Cat B Enz* 2001, **13**:37-47.

27. • Kronenburg NAE, de Bont JAM, Fischer L: **Improvement of enantioselectivity by immobilized imprinting of epoxide hydrolase from *Rhodotorula glutinis***. *J Mol Cat B Enz* 2001, **16**:121-129.
Reversal of enantiopreference was the result of imprinting the enzyme with the least preferred enantiomer during the immobilization process.

28. Laughlin LT, Tzeng HF, Lin S, Armstrong RN: **Mechanism of microsomal epoxide hydrolase. Semifunctional site-specific mutants affecting the alkylation half-reaction**. *Biochemistry* 1998, **37**:2897-2904.

29. Ichiyama S, Kurihara T, Miyagi M, Galkin A, Tsunasawa S, Kawasaki H, Esaki N: **Catalysis-linked inactivation of fluoroacetate dehalogenase by ammonia: a novel approach to probe the active-site environment**. *J Biochem* 2002, **131**:671-677.

30. Kolattukudy PE, Brown L: **Fate of naturally occurring epoxy acids: a soluble epoxide hydrase, which catalyzes *cis* hydration, from *Fusarium solani pisi***. *Arch Biochem Biophys* 1975, **166**:599-607.

31. Haeggstrom JZ, Kull F, Rudberg PC, Tholander F, Thunnissen MM: **Leukotriene A4 hydrolase**. *Prostaglandins Other Lipid Mediat* 2002, **68-69**:495-510.

32. Barbirato F, Verdoes JC, de Bont JA, van der Werf MJ: **The *Rhodococcus erythropolis* DCL14 limonene-1,2-epoxide hydrolase gene encodes an enzyme belonging to a novel class of epoxide hydrolases**. *FEBS Lett* 1998, **438**:293-296.

33. Muller F, Arand M, Frank H, Seidel A, Hinz W, Winkler L, Hanel K, Blee E, Beetham JK, Hammock BD, Oesch F: **Visualization of a covalent intermediate between microsomal epoxide hydrolase, but not cholesterol epoxide hydrolase, and their substrates**. *Eur J Biochem* 1997, **245**:490-496.

34. • Van Hylckama Vlieg JE, Tang L, Lutje Spelberg JH, Smilda T, Poelarends GJ, Bosma T, van Merode AE, Fraaije MW, Janssen DB: **Halohydrin dehalogenases are structurally and mechanistically related to short-chain dehydrogenases/reductases**. *J Bacteriol* 2001, **183**:5058-5066.

A mechanism of the haloalcohol dehalogenases was proposed, based on the structural analogy with SDR proteins.

35. Assis HM, Sallis PJ, Bull AT, Hardman DJ: **Biochemical characterization of a haloalcohol dehalogenase from *Arthrobacter erithii* H10a**. *Enz Microb Tech* 1998, **22**:568-574.

36. Kasai N, Suzuki T, Furukawa Y: **Chiral C3 epoxides and halohydrins: their preparation and synthetic application**. *J Mol Cat B Enz* 1998, **4**:237-252.

37. Nakamura T, Nagasawa T, Yu F, Watanabe I, Yamada H: **A new catalytic function of halohydrin hydrogen halide lyase, synthesis of β-hydroxynitriles from epoxides and cyanide**. *Biochem Biophys Res Commun* 1991, **180**:124-130.

38. •• Lutje Spelberg JH, Tang LX, van Gelder M, Kellogg RM, Janssen DB: **Exploration of the biocatalytic potential of a halohydrin dehalogenase using chromogenic substrates**. *Tetrahedron Asymmetry* 2002, **13**:1083-1089.

In this article (together with [39]) the range of accepted nucleophiles, the regioselectivity, and enantioselectivity of conversion of epoxides catalysed by haloalcohol dehalogenase is described.

39. Lutje Spelberg JH, van Hylckama Vlieg JE, Tang L, Janssen DB, Kellogg RM: **Highly enantioselective and regioselective biocatalytic azidolysis of aromatic epoxides**. *Org Lett* 2001, **3**:41-43.

40. De Jong RM, Rozeboom HJ, Kalk KH, Tang L, Janssen DB, Dijkstra BW: **Crystallization and preliminary X-ray analysis of an enantioselective halohydrin dehalogenase from *Agrobacterium radiobacter* AD1**. *Acta Crystallogr D* 2002, **58**:176-178.

41. Laughlin LT, Bernat BA, Armstrong RN: **Mechanistic imperative for the evolution of a metalloglutathione transferase of the vicinal oxygen chelate superfamily**. *Chem Biol Interact* 1998, **111-112**:41-50.

42. Bogaards JJ, Venekamp JC, Salmon FG, van Bladeren PJ: **Conjugation of isoprene monoepoxides with glutathione, catalyzed by alpha, mu, pi and theta-class glutathione S-transferases of rat and man**. *Chem Biol Interact* 1999, **117**:1-14.

43. Wistuba D, Weigand K, Peter H: **Stereoselectivity of *in vitro* isoprene metabolism**. *Chem Res Toxicol* 1994, **7**:336-343.

44. Smoukov SK, Telser J, Bernat BA, Rife CL, Armstrong RN, Hoffman BM: **EPR study of substrate binding to the Mn(II) active site of the bacterial antibiotic resistance enzyme FosA: a better way to examine Mn(II)**. *J Am Chem Soc* 2002, **124**:2318-2326.

45. Bernat BA, Laughlin LT, Armstrong RN: **Regiochemical and stereochemical course of the reaction catalyzed by the fosfomycin resistance protein, FosA**. *J Org Chem* 1998, **63**:3778-3780.

46. Cao M, Bernat BA, Wang Z, Armstrong RN, Helmann JD: **FosB, a cysteine-dependent fosfomycin resistance protein under the control of σ^W, an extracytoplasmic-function sigma factor in *Bacillus subtilis***. *J Bacteriol* 2001, **183**:2380-2383.

47. Clark DD, Allen JR, Ensign SA: **Characterization of five catalytic activities associated with the NADPH:2-ketopropyl-coenzyme M [2-(2-ketopropylthio)ethanesulfonate] oxidoreductase/carboxylase of the *Xanthobacter* strain Py2 epoxide carboxylase system**. *Biochemistry* 2000, **39**:1294-1304.

48. Allen JR, Ensign SA: **Two short-chain dehydrogenases confer stereoselectivity for enantiomers of epoxypropane in the multiprotein epoxide carboxylating systems of *Xanthobacter* strain Py2 and *Nocardia corallina* B276**. *Biochemistry* 1999, **38**:247-256.

49. Krum JG, Ellsworth H, Sargeant RR, Rich G, Ensign SA: **Kinetic and microcalorimetric analysis of substrate and cofactor interactions in epoxyalkane:CoM transferase, a zinc-dependent epoxidase**. *Biochemistry* 2002, **41**:5005-5014.

50. Allen JR, Clark DD, Krum JG, Ensign SA: **A role for coenzyme M (2-mercaptoethanesulfonic acid) in a bacterial pathway of aliphatic epoxide carboxylation**. *Proc Natl Acad Sci USA* 1999, **96**:8432-8437.

51. Weijers CAGM, De Haan A, De Bont JAM: **Chiral resolution of 2,3-epoxyalkanes by *Xanthobacter* Py2**. *Appl Microbiol Biotech* 1988, **27**:337-340.

52. Clark DD, Allen JR, Ensign SA: **Characterization of five catalytic activities associated with the NADPH:2-ketopropyl-coenzyme M [2-(2-ketopropylthio)ethanesulfonate] oxidoreductase/carboxylase of the *Xanthobacter* strain Py2 epoxide carboxylase system**. *Biochemistry* 2000, **39**:1294-1304.

53. Arand M, Hallberg BM, Zou J, Bergfors T, Oesch F, van der Werf MJ, de Bont JA, Jones TA, Mowbray SL: **Structure of *Rhodococcus erythropolis* limonene-1,2-epoxide hydrolase reveals a novel active site**. *EMBO J* 2003, **22**:2583-2592.

54. Tang L, Lutje Spelberg JH, Fraaije MW, Janssen DB: **Kinetic mechanism and enantioselectivity of halohydrin dehalogenase from *Agrobacterium radiobacter***. *Biochemistry* 2003, **42**:5378-5386.