Appl Microbiol Biotechnol (1988) 27:337–340

Applied and Microbiology Biotechnology

Chiral resolution of 2,3-epoxyalkanes by *Xanthobacter* Py2

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Summary. With propene-grown cells of *Xanthobacter* Py2 it was possible to resolve racemic mixtures of 2,3-epoxyalkanes. Only 2*S*-forms were metabolized by this organism, resulting in pure 2*R*-2,3-epoxyalkanes. Chiral resolution was obtained with *trans*-2,3-epoxybutane, *trans*-2,3-epoxypentane and *cis*-2,3-epoxypentane. *Xanthobacter* Py2 was however not able to discriminate between the enantiomeric forms of 1,2-epoxyalkanes, resulting in the complete degradation of both chiral forms of 1,2-epoxyalkanes.

Introduction

Epoxides are valuable starting materials in organic synthesis and they are used on a large scale in chemical industry as precursors of many different compounds. Epoxides are of even greater interest when available in optically pure form because they then can serve as synthons in the preparation of other more valuable optically active compounds. Synthesis of chiral epoxides by chemical methods has been studied extensively (Scott 1984), but apart from the asymmetric epoxidation of allylic alcohols (Katsuki 1980) and apart from the formation of some other chiral epoxides as for instance 1,2-epoxypropane (Golding 1985) no general procedure is available for the selective formation of the respective enantiomeric forms of epoxides. Consequently, many attempts have been made in recent years to produce chiral epoxides from alkenes by biological methods. Bacteria have been described that are able to produce chiral epoxyalkanes from gaseous alkenes (Furuhashi et al. 1981; Habets-Crützen et al. 1985), from higher alkenes (de Smet et al. 1983) and from more complex alkenes as for instance arylallyl ethers (Johnstone et al. 1986). Prospects and limitations of such systems that all depend on the action of mono-oxygenases are presently studied by several research groups (Tramper et al. 1984; Drozd and Balley 1984).

However, another biological method to obtain optically pure epoxides may be available by using enzymes that degrade epoxides stereoselectively. Such method would involve the complete degradation of one stereo isomer while the other isomer would not be affected. Although resolution of epoxides in this manner involves the destruction of half of the amount of epoxide, it nevertheless may be an economically feasible method in view of the difference in price between the racemic and optically pure epoxides. Enantioselective hydrolysis has previously been observed for microsomal epoxide hydrolase (Seidegard and DePierre 1983), and the conversion of epoxides into *trans*-diols by this enzyme has recently been reviewed by Berti (1986) from the point of view of the relations between substrate structure and the regio- and stereoselectivity of the enzyme.

Recently we have screened several epoxide-degrading bacteria for their ability to degrade epoxyalkanes stereoselectively and the effect of these strains on racemic mixtures of both 1,2-epoxyalkanes and 2,3-epoxyalkanes has been investigated. In the present paper we report on the results obtained with *Xanthobacter* Py2 for the stereoselective degradation of epoxyalkanes. The organism was originally isolated on propene (Habets-Crützen et al. 1984) and it also grows on other 1-alkenes (ethene, 1-butene) as well as on 1,2-epoxyalkanes (1,2-epoxypropane, 1,2-epoxybutane) (van Ginkel et al. 1986). It contains an alkene mono-oxygenase that oxidizes 1-alkenes to

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R-1,2-epoxyalkanes (Habets-Crützen et al. 1985). It is now shown that the organism contains an enzyme involved in the further metabolism of epoxyalkanes that is able to discriminate between the enantiomeric forms of 2,3-epoxyalkanes.

Materials and methods

Organism and culture conditions. Xanthobacter Py2 has been described previously by van Ginkel and de Bont (1986). The organism was cultivated continuously in mineral medium in a 3-litre fermentor (with 2 litre working volume) at 30° C, with a dilution rate of 0.02 h^{-1}. The pH of the culture was maintained at 7.0. As carbon source propene was supplied as a 100 ml/min 4% in air mixture. The cells were harvested by centrifugation at 16,000 *g*, washed twice with 50 mM potassium phosphate buffer pH 7.0 and stored at −20°C.

Chemicals. Gaseous alkenes were obtained from Hoek Loos, Schiedam (NL). Other alkenes, 1,2-epoxyalkanes, *trans*-2,3-epoxybutane and *cis*-2,3-epoxybutane were from Aldrich Chemie N.V., Brussels. *Trans*-2,3-epoxypentane and *cis*-2,3-epoxypentane were synthesized by oxidation of the corresponding 2-pentenes with *m*-chloroperoxybenzoic acid (Swern et al. 1946).

Analysis of epoxyalkanes. The characterization of the synthesized epoxypentanes was carried out by NMR spectroscopy and mass spectroscopy. ^{1}H-NMR spectra were recorded on a Varian EM-390 90 MHz spectrometer and mass spectra on a Vacuum Generators Ltd. Micromass 7070F Mass-spectrometer.

The complexation g.l.c. method described by Schurig and Bürkle (1982) was used to determine the enantiomeric composition of the various epoxyalkanes. Analyses were carried out on a Packard model 438 gas chromatograph. As carrier gas N_2 was used and the oven temperature was 50° C. A glass capillary column was used: Length 25 m, diameter 0.25 mm, coated with 'Chirametal-27-*R*-3-1-13'. This column was obtained from CC & CC, P.O. Box 14, D-7402 Kirchentellinsfurt, FRG. Peak areas were determined with a Shimadzu model Chromatopac C-R3A integrator.

Degradation of epoxyalkanes by Xanthobacter Py2. Epoxyalkane degradation by *Xanthobacter* Py2 was tested in 27 cm^3 screw-cap bottles sealed with rubber septa. Bottles contained 4 ml 50 mM potassium phosphate buffer with the appropriate epoxyalkanes and 0.05 ml methane serving as internal standard. The bottles were placed into a shaking waterbath (30° C, 3 Hz) and the reaction was started by injecting 1 ml washed cell-suspension (25 mg protein). Periodically 100 µl samples of the gas phase were taken and analysed by complexation g.l.c. Concentrations of epoxyalkanes were derived from calibration curves using heat killed cells. Epoxyalkane concentrations are expressed in mM in the water-phase assuming the quantity of epoxyalkane in the gas-phase is negligible in comparison with the quantity of epoxyalkane in the waterphase.

Table 1. Degradation of epoxyalkanes by propene-grown *Xanthobacter* Py2

Substrate	Degradation rate[a]
1,2-Epoxypropane	35
1,2-Epoxybutane	32
Cis-2,3-epoxybutane	10
Trans-2,3-epoxybutane	6
Cis-2,3-epoxypentane	9
Trans-2,3-epoxypentane	4
1,2-Epoxyhexane	15

[a] Rates are expressed in nmol per minute per mg protein

Results and discussion

Epoxyalkane utilization by Xanthobacter Py2

Xanthobacter Py2, when growing on an alkene, in an at present unknown type of reaction further degrades epoxyalkanes to eventually cell biomass and carbon dioxide (van Ginkel and de Bont 1986). Washed cell suspensions of propene-grown cells metabolize several epoxyalkanes and the initial rates of degradation of various racemic epoxyalkane mixtures are given in Table 1. From these results it appears that an epoxide-degrading enzyme with a broad substrate specificity is present in propene-grown *Xanthobacter* Py2 cells, although it can not be excluded that more than one enzyme system is involved in the metabolism of the various epoxyalkanes.

Stereoselectivity of epoxyalkane degradation

The stereoselectivity of the epoxide-degrading enzyme in *Xanthobacter* Py2 was tested by incubating washed propene-grown cells with racemic mixtures of 1,2-epoxypropane and *trans*-2,3-epoxybutane respectively. Enantiomeric resolution of the epoxyalkanes was followed by taking samples from head space and analysis by complexation gas chromatography (Schurig and Bürkle 1982). No clear preferential utilization of one of the configurations of 1,2-epoxypropane was observed; both isomers were fully degraded (Fig. 1a). Interestingly, a complete different situation was met with *trans*-2,3-epoxybutane. In this case only the 2*S*,3*S*-isomer was degraded while the 2*R*,3*R*-isomer was not metabolized at all (Fig. 1b). Furthermore, no effect of the 2*R*,3*R*-isomer on the degradation of the 2*S*,3*S*-isomer was observed since an extra addition of racemic *trans*-2,3-epoxybutane, after resolution of the initial mixture, did not affect the rate of degradation of the 2*S*,3*S*-isomer (Fig. 2). In another experiment was the initial concentration of the racemic *trans*-2,3-

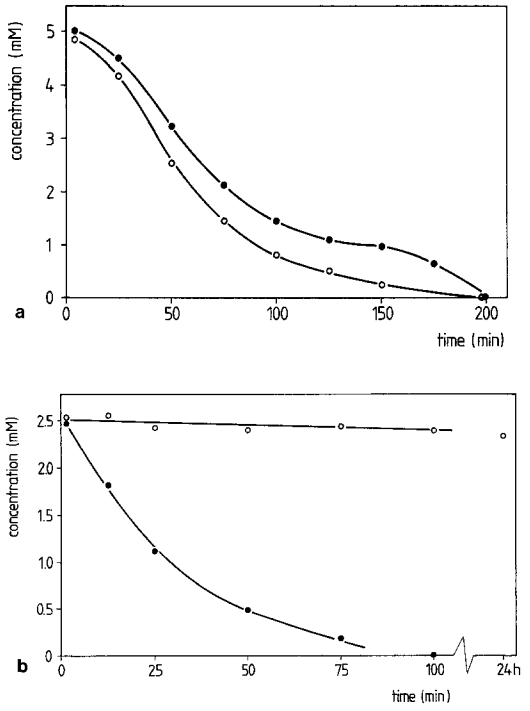

Fig. 1. Degradation of epoxyalkanes by propene-grown *Xanthobacter* Py2. **(a)** Simultaneous degradation of *R*-1,2-epoxypropane (○) and *S*-1,2-epoxypropane (●); **(b)** degradation of *trans*-(2*S*,3*S*)-epoxybutane (●), *trans*-(2*R*,3*R*)-epoxybutane (○) was not degraded

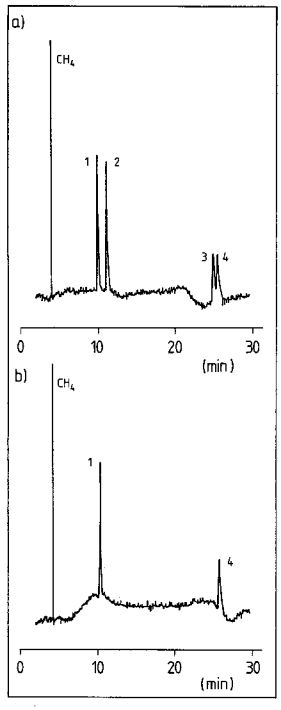

Fig. 3. Chromatograms of 2,3-epoxypentanes as obtained by complexation gas chromatography. *Trans*-(2*R*,3*R*)-epoxypentane *(1)*, *trans*-(2*S*,3*S*)-epoxypentane *(2)*, *cis*-(2*S*,3*R*)-epoxypentane *(3)* and *cis*-(2*R*,3*S*)-epoxypentane *(4)*; **(a)** initial mixture, **(b)** mixture remaining after treatment with *Xanthobacter* Py2

epoxybutane raised to 100 mM and under that condition was also a complete resolution of the mixture obtained. In this way it was possible to obtain in a very simple way the pure *trans*-(2*R*,3*R*)-epoxybutane whereas the chemical method for the preparation of this chiral compound from (2*S*,3*S*)-tartaric acid involves eight different steps (Schurig et al. 1980).

Cis-2,3-epoxybutane is not a chiral compound and it was degraded to completion by washed-cell suspensions of propene-grown *Xanthobacter* Py2.

Investigations were further extended by preparing racemic 2,3-epoxypentanes from both *cis*-2-pentene and from *trans*-2-pentene and the effect of *Xanthobacter* Py2 cells on the resulting four epoxyalkanes was recorded. The racemic mixtures of both the *trans* and the *cis* form were given in separate experiments to the washed cells. Both mixtures were resolved and the remaining enantiomers were *trans*-(2*R*,3*R*)-epoxypentane and *cis*-(2*R*,3*S*)-epoxypentane respectively. In another experiment were both racemic mixtures given simultaneously to the cells again resulting in the degradation of the 2*S*-forms and in an accumulation of the 2*R*-forms (Fig. 3).

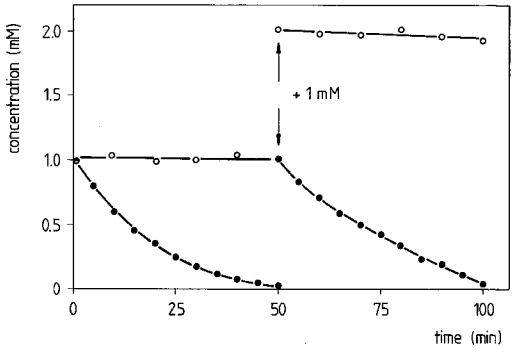

Fig. 2. Effect of *trans*-(2*R*,3*R*)-epoxybutane (○) on the degradation of *trans*-(2*S*,3*S*)-epoxybutane (●) by *Xanthobacter* Py2

Conclusions

From the results presented it is concluded that *Xanthobacter* Py2 cells are able to discriminate between the enantiomeric forms of 2,3-epoxyalkanes (Fig. 4) whereas both chiral forms of 1,2-epoxyalkanes are fully degraded. At present it re-

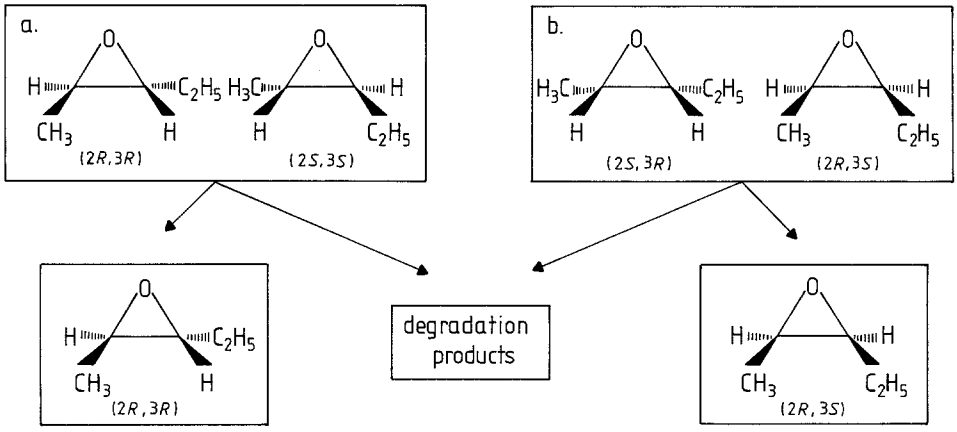

Fig. 4. Racemic mixtures of **(a)** *trans*-2,3-epoxypentane, **(b)** *cis*-2,3-epoxypentane and the remaining isomers after chiral resolution by *Xanthobacter* Py2

mains uncertain whether only one enzyme is involved in these epoxide-degrading reactions, but it nevertheless is obvious that using *Xanthobacter* Py2 it is possible to resolve racemic mixtures of 2,3-epoxyalkanes. This stereoselective degradation of epoxyalkanes appears to be a very elegant way to obtain an epoxyalkane in an enantiomerically pure form and we therefore at present are studying the epoxide-degrading enzyme of *Xanthobacter* Py2 involved in the resolution of 2,3-epoxyalkanes and we also are testing other organisms for the resolution of 1,2-epoxyalkanes.

Acknowledgements. We are grateful to Mr. B. J. M. Jansen for his advice in the synthesis of the epoxypentanes and to Dr. M. A. Posthumus for performing the mass spectrum analyses.

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Received June 19, 1987/Accepted August 5, 1987