Appl Microbiol Biotechnol (1992) 38:297-300

Applied and Microbiology Biotechnology

© Springer-Verlag 1992

Synthesis of optically pure 1,2-epoxypropane by microbial asymmetric reduction of chloroacetone

C. A. G. M. Weijers, M. J. J. Litjens, J. A. M. de Bont

Division of Industrial Microbiology, Department of Food Science, Agricultural University Wageningen, P. O. Box 8129, NL-6700 EV Wageningen, The Netherlands

Received: 19 May 1992/Accepted: 20 July 1992

Abstract. A number of bacteria and yeasts was screened for asymmetric reduction of prochiral chloroacetone into chiral 1-chloro-2-propanol, which is chemically convertible into chiral 1,2-epoxypropane. In this way *Rhodotorula glutinis* produced optically pure *S*-1,2-epoxypropane with 98% enantiomeric excess and in a relatively high final concentration. The enzyme that catalysed the asymmetric reduction was an NAD(P)H-dependent alcohol dehydrogenase. Reduction of racemic 3-chloro-2-butanone resulted in mixtures of *cis* and *trans*-2,3-epoxybutane, indicating that no enantioselective reduction of this haloketone occurred.

Introduction

A great interest exists in the development of methods for the synthesis of optically pure epoxyalkanes, because they are very important chiral building blocks that can serve as synthons in the preparation of more complex optically pure bioactive compounds (de Smet et al. 1983; Furuhashi 1986). Chiral 1,2-epoxypropane has served as a model compound in biological production of optically pure epoxyalkanes and most studies have been based on stereospecific epoxidation of alkenes by monooxygenase-containing bacterial cells (Furuhashi et al. 1981; Hou et al. 1983; Habets-Crützen et al. 1985; Subramanian 1986; Weijers et al. 1988a). In all these cases 1,2-epoxypropane was produced either as a racemic mixture or with the *R* configuration in excess. The method was problematic due to product toxicity (Habets-Crützen and de Bont 1985). In another microbiological method, optically pure epoxyalkanes were enriched from racemic epoxyalkanes by kinetic resolution. *Nocardia* H8 degraded the *R* and *S* enantiomers of 1,2-epoxypropane at different rates, which allowed the production of *S*-1,2-epoxypropane in optically pure form (Weijers and de Bont 1991). However, the reaction yield was low.

Correspondence to: C. A. G. M. Weijers

In view of the disadvantages of the existing methods, we have now investigated an alternative production route, which is based on the ability of many microorganisms to perform asymmetric reduction of haloketones. The resulting optically pure α-halohydrins may subsequently be chemically converted into optically pure epoxides. Microbial asymmetric ketone reduction as a method for the production of chiral alcohols has been studied extensively (Sih and Chen 1984). Most of the literature is based on studies with baker's yeast as the biocatalyst (Servi 1990; Ward and Young 1990) and far less attention has been given to other yeast strains, to fungi (Nakamura et al. 1989; Fauve and Veschrambre 1990) or to bacteria (Keinan et al. 1986; Maconi and Aragozzini 1989; Adlercreutz 1991). Microbial reduction of α-haloketones has been reported for only a very limited number of substrates and organisms. Reduction of α-haloarylketones with *Saccharomyces cerevisiae* and *Cryptococcus macerans* (Carvalho et al. 1991; Imuta et al. 1980) and reduction of chlorolactic acid with lactic dehydrogenase from *Lactobacillus leichmannii* (Hirschbein and Whitesides 1982) have been described.

In the present study prochiral chloroacetone was microbially reduced into chiral 1-chloro-2-propanol from which chiral 1,2-epoxypropane was chemically obtained (Fig. 1). A number of yeasts and bacteria was screened and the yeast *Rhodotorula glutinis* was selected to study this reaction in more detail.

Materials and methods

Microorganisms and growth conditions. The microorganisms tested for the asymmetric reduction of chloroacetone were from different culture collections. Bacteria and yeasts were obtained from the American Type Culture Collection (ATCC), USA from the Centraalbureau voor Schimmelcultures (CBS), the Netherlands, from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM), FRG and from our own laboratory culture collection.

During screening, lactic acid bacteria were batch-grown in MRS medium (de Man et al. 1960) while the other bacteria were cultivated in a mineral medium with 0.2% (w/w) yeast extract,

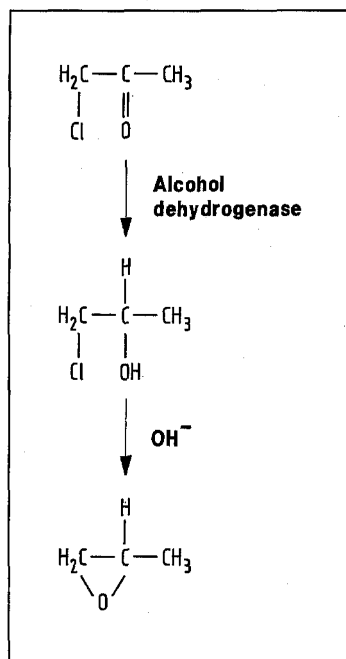

Fig. 1. Biological reduction of chloroacetone to 1-chloro-2-propanol and chemical conversion of the halohydrin into 1,2-epoxypropane

supplemented with 0.2% (v/v) 2-propanol as the carbon source. Yeasts were cultivated in the corresponding medium with 1% (w/w) glucose as carbon source. All strains were incubated at 30° C on a rotary shaker, harvested in the late log phase by centrifugation at 16000 *g*, washed twice with 50 mM potassium phosphate buffer, pH 7.0, concentrated, and stored at −20° C.

Chemicals. Chloroacetone, 1-bromo-2-butanone, 3-chloro-2-butanone and 1-chloro-2-propanol were obtained from Aldrich (Brussels, Belgium). 1,2-Epoxypropane, 1,2-epoxybutane and *cis*-2,3-epoxybutane and *trans*-2,3-epoxybutane were from Merck (Darmstadt, FRG).

Analytical methods. Haloketone concentrations were determined by headspace analysis on a Carlo Erba model 4200 gas chromatograph fitted with a capillary Poraplot Q column (Chrompack, The Netherlands). As carrier gas N_2 was used and the oven temperature was 170° C. Analyses of 1-chloro-2-propanol together with chloroacetone were carried out by injecting 1-μl liquid samples into a Chrompack Packard model 437A gas chromatograph fitted with a 20% Tween 80-Chromosorb W-AW column at an oven temperature of 145° C and with N_2 as carrier gas. Detections were in both techniques with flame ionization detectors. The complexation gas-liquid chromatography (GLC) method of Schurig and Bürkle (1982) was used to determine the enantiomeric composition of the epoxyalkanes as also described in our previous studies (Weijers et al. 1988a, b).

Chloroacetone reduction and 1,2-epoxypropane formation with whole cells. Reduction of chloroacetone and the other haloketones was tested in 40 cm^3 screw-capped bottles sealed with rubber septa. The bottles contained 4 ml of 50 mM potassium phosphate buffer, pH 7.0, and 1 ml concentrated washed cell suspension (20 mg protein) of the selected microorganism. The bottles were placed into a shaking water bath at 30° C and the reaction was started by addition of 50 μmol of the appropriate haloketone substrate and 0.05 ml methane serving as the internal standard. The decrease in haloketone concentration was followed by head-space analysis during the course of the reduction reaction. After completion of the reaction, 100 μl of 5 M potassium hydroxide was added to the reaction mixture for conversion of the halohydrin formed to the epoxyalkane. Eventually, the enantiomeric composition of the epoxyalkane was determined by head-space analysis with complexation GLC. The detailed bioconversion of chloroacetone into 1-chloro-2-propanol with cells of *R. glutinis* was performed in 120-ml bottles with a 15-ml reaction volume. This specific reaction was followed by periodically taking samples from the reaction mixture, centrifuging for 2 min at 15000 *g* and analysing the supernatants by gas chromatography. The concentrations of haloketones, halohydrin and epoxyalkanes were derived from calibration curves using heat-killed cells.

Enzyme preparations. Crude cell-free extracts were obtained after ultrasonic disintegration of concentrated *R. glutinis* cells followed by removal of cell debris and unbroken cells by centrifugation at 30000 *g* for 20 min at 4° C. The fraction of crude extract precipitating between 55% and 75% ammonium sulphate saturation was collected by centrifugation, dissolved in 20 mM TRIS-HCl buffer, pH 7.5, and dialysed overnight at 4° C against the same buffer. Further purification was performed with fast protein liquid chromatography (FPLC) using a Mono Q (HR 5/5) column equilibrated with TRIS-HCl buffer, pH 7.5. The protein was eluted with a gradient of 0–1 M NaCl in the same buffer. Fractions containing activity were pooled and dialysed for removal of NaCl. All purification steps were performed at 4° C.

Enzyme assay. Alcohol dehydrogenase activity was measurd spectrophotometrically at 340 nm with NAD(P)H as the electron donor. The reaction mixture, in a total volume of 1.0 ml, contained 20 mM TRIS-HCl buffer, pH 7.5, enzyme preparation and 0.2 μmol NAD(P)H. The reaction was started by addition of 0.1 mmol chloroacetone and the rate of NAD(P)H oxidation at 30° C was measured.

Synthesis of 1,2-epoxypropane by reduction of chloroacetone with the enzyme preparations was performed as with whole cells but with supplementary addition of NAD(P)H and the gas chromatography analyses were only with head-space samples.

Results and discussion

Asymmetric reduction of chloroacetone by various microorganisms

Yeasts and bacteria that were able to reduce chloroacetone were tested for their ability to reduce the compound asymmetrically (Table 1). Reduction by the bacterial strains resulted either in racemic 1,2-epoxypropane or in 1,2-epoxypropane with a low degree of optical purity in the *R*-configuration. However, the two heterofermenting lactic acid bacteria yielded the *S*-enantiomer and even in very high optical purity with *Leuconostoc paramesenteroides*. The enantiospecificity of these and other lactic acid bacteria had already been observed in the asymmetric reduction of some other, non-halogenated ketones (Maconi and Aragozzini 1989).

1,2-Epoxypropane obtained with the various yeast strains was racemic or mainly in the *S*-configuration. High degrees of optial purity of up to 98% enantiomeric excess (ee) were obtained with *S. cerevisiae* and *R. glutinis*. The asymmetric reduction reaction was investigated in more detail in *R. glutinis* since this strain had the highest reaction rate (data not shown) and since the organism was simple to grow.

Table 1. Formation of 1,2-epoxypropane by chloroacetone reduction with several selected bacteria and yeasts

Microorganism	1,2-Epoxy propane	
	% ee	Abs. conf.
Brevibacterium butanicum ATCC 21196	60	*R*
Corynebacterium alkanum ATCC 21194	74	*R*
C. hydrocarboclastass ATCC 15108	28	*R*
Lactobacillus brevis DSM 1267	24	*S*
Leuconostoc paramesenteroides DSM 20288	98	*S*
Mycobacterium vaccae ATCC 29678	8	*R*
Nocardia butanica ATCC 21197	0	–
N. paraffinica ATCC 21198	76	*R*
Nocardia sp. EE1	58	*R*
Nocardia sp. H8	18	*R*
Nocardia sp. IP1	0	–
Nocardia sp. TB1	60	*R*
Xanthobacter sp. Py2	4	*R*
Candida tropicalis CBS 573	48	*R*
C. utilis CBS 621	0	–
Hansenula capsulata DSM 70269	84	*S*
Kluyveromyces marxianus CBS 6556	32	*R*
Pichia pastoris	4	*S*
Rhodotorula glutinis	98	*S*
R. graminis	92	*S*
R. minuta	72	*S*
Saccharomyces cerevisiae CBS 1242	74	*S*
S. cerevisiae CBS 1394	98	*S*
S. ludwigii	86	*S*
Schizosaccharomyces octosporus	60	*S*
Yarrowia lipolytica	74	*S*

ee, enantiomeric excess; abs. conf., absolute configuration

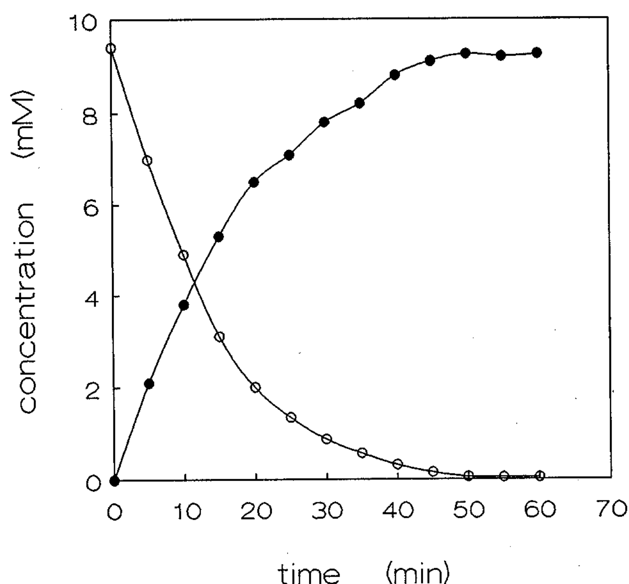

Fig. 2. Time course of the reduction of chloroacetone (O) into 1-chloro-2-propanol (●) with glucose-grown cells of *Rhodotorula glutinis*. The reaction mixture (15 ml) contained 70 mg protein

Asymmetric reduction of haloketones by R. glutinis

For future application and optimization of the production method of 1,2-epoxypropane, the reductive reaction step was studied in more detail. In Fig. 2 it is demonstrated that cells of *R. glutinis* perform a quantitative conversion of chloroacetone into 1-chloro-2-propanol. The enzyme involved in this reaction was found to be an NAD(P)H-dependent alcohol dehydrogenase. The enzymatic reaction was studied in crude cell-free extracts of *R. glutinis* and also in partially purified fractions obtained after ammonium sulphate precipitation and FPLC. The enantiospecificity in the successive enzyme fractions was in accordance with the results obtained in whole cells. Therefore, it is concluded that only one type of alcohol dehydrogenase with high enantiospecificity is involved in the asymmetric reduction of chloroacetone by *R. glutinis*.

Enantiospecificity was also studied in the formation of two other chiral epoxyalkanes by *R. glutinis* cells (Table 2). Reduction of prochiral 1-bromo-2-butanone resulted in optically pure *S*-1,2-epoxybutane, but reduction of racemic 3-chloro-2-butanone gave two stereoisomers of 2,3-epoxybutane. Optically pure *trans*-(2*S*,3*S*)-epoxybutane and the non-chiral (*meso*) *cis*-2,3-epoxybutane were formed in nearly equal amounts. This result indicates that both enantiomers of racemic 3-chloro-2-butanone were reduced with nearly equal reaction rates. Because the *trans*-2,3-epoxybutane product consisted of only the 2*S*,3*S*-enantiomer we conclude that 3*R*-chloro-2-butanone was asymmetrically reduced to (3*R*,2*S*)-3-chloro-2-butanol, which was chemically converted into *trans*-2*S*,3*S*-epoxybutane. Similarly the reduction of 3*S*-chloro-2-butanone gave (3*S*,2*S*)-3-chloro-2-butanol and the conversion to *cis*-2,3-epoxybutane. With the other tested yeasts and bacteria, formation of 2,3-epoxybutane in all cases resulted in mixtures of the *cis* and *trans* stereoisomers, demonstrating that no organism reduced the haloketone enantioselectively. From this it can be concluded that reduction of racemic aliphatic haloke-

Table 2. Formation of 1,2-epoxypropane and epoxybutanes by haloketone reduction with glucose-grown cells of *R. glutinis*

Haloketone substrate	Reduction rate[a]	Epoxyalkane product	ee (%)	Abs. conf.	Yield (%)
Chloroacetone	48	1,2-Epoxypropane	98	*S*	96
1-Bromo-2-butanone	58	1,2-Epoxybutane	98	*S*	93
3-Chloro-2-butanone	106	*cis*-2,3-Epoxybutane	—	—	48
		trans-2,3-Epoxybutane	98	*S, S*	49

[a] Rates are expressed in nmoles per minute per milligram protein

tones is not an appropriate method for the production of optically pure 2,3-epoxyalkanes. Therefore, at this moment the most successful reported method for microbial formation of optically pure 2,3-epoxyalkanes remains the kinetic resolution of racemic mixtures with *Xanthobacter* Py2 (Weijers et al. 1988b).

With *R. glutinis* the present method proceeds with moderate reduction rates for the haloketones and with good reaction yield up to 96% (Table 2). In comparison with the epoxidation of alkenes (Habets-Crützen and de Bont 1985) this method suffers much less from product toxicity towards the microorganism and final concentration of up to 100 mM α-halohydrin and 1,2-epoxyalkane were easily achieved. Yields of 1,2-epoxyalkane formation by α-haloketone reduction are higher than by the method of kinetic resolution of racemic 1,2-epoxyalkanes. With the latter method the maximum yield is 50% and in the described enrichment of optically pure *S*-1,2-epoxypropane by *Nocardia* H8 the yield was only 13%, due to low enantioselectivity of the involved epoxyalkane-degrading enzyme (Weijers and de Bont 1991). Consequently, asymmetric α-haloketone reduction appears to be a promising alternative method for microbial formation of optically pure 1,2-epoxyalkanes.

References

Adlercreutz P (1991) Novel biocatalyst for the asymmetric reduction of ketones: permeabilized cells of *Gluconobacter oxydans*. Enzyme Microb Technol 13:9–14

Carvalho M de, Okamoto MT, Moran PJS, Rodrigues AR (1991) Baker's yeast reduction of α-haloacetophenones. Tetrahedron 47:2073–2080

Fauve A, Veschambre H (1990) Regiospecificity and enantiospecificity in microbiological reduction of acylic β-diketones. Biocatalysis 3:95–109

Furuhashi K (1986) A fermentation process for the production of optically active epoxides. Chem Economy Eng Rev 18:21–26

Furuhashi K, Taoka A, Uchida S, Karube I, Suzuki S (1981) Production of 1,2-epoxyalkanes from 1-alkenes by *Nocardia corallina* B-276. Eur J Appl Microbiol Biotechnol 12:39–45

Habets-Crützen AQH, Bont JAM de (1985) Inactivation of alkene oxidation by epoxides in alkene- and alkane-grown bacteria. Appl Microbiol Biotechnol 22:428–433

Habets-Crützen AQH, Carlier SJN, Bont JAM de, Wistuba D, Schurig V, Hartmans S, Tramper J (1985) Stereospecific formation of 1,2-epoxypropane, 1,2-epoxybutane and 1-chloro-2,3-epoxypropane by alkene-utilizing bacteria. Enzyme Microbiol Technol 7:17–21

Hirschbein BL, Whitesides GM (1982) Laboratory-scale enzymatic/chemical synthesis of D- and L-β-chlorolactic acid and D- and L-potassium glycidate. J Am Chem Soc 104:4458–4460

Hou CT, Patel R, Laskin AI, Barnabe N, Barist I (1983) Epoxidation of short-chain alkenes by resting-cell suspensions of propane-grown bacteria. Appl Environ Microbiol 46:171–177

Imuta M, Kawai K, Ziffer H (1980) Product stereospecificity in the microbial reduction of α-haloaryl ketones. J Org Chem 45:3352–3355

Keinan E, Hafeli EK, Seth KK, Lamed R (1986) Thermostable enzymes in organic synthesis. Asymmetric reduction of ketones with alcohol dehydrogenase from *Thermoanaerobium brockii*. J Am Chem Soc 108:162–169

Maconi E, Aragozzini F (1989) Stereoselective reduction of noncyclic ketones with lactic acid bacteria. Appl Microbiol Biotechnol 31:29–31

Man JC de, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130–135

Nakamura K, Kawai Y, Kitayama T, Miyai T, Ogawa M, Mikata Y, Higaki M, Ohno A (1989) Asymmetric reduction of ketones with microbes. Bull Inst Chem Res Kyoto Univ 67:157–168

Servi S (1990) Baker's yeast as a reagent in organic synthesis. Synthesis 1:1–25

Sih CJ, Chen CS (1984) Mikrobielle asymmetrische Katalyse. Enantioselektive Reduktion von Ketonen. Angew Chem 96:556–565

Schurig V, Bürkle W (1982) Extending the scope of enantiomer resolution by complexation gas chromatography. J Am Chem Soc 104:7573–7580

Smet de MJ, Kingma J, Wynberg H, Witholt B (1983) *Pseudomonas oleovorans* as a tool in bioconversions of hydrocarbons: growth, morphology and conversion characteristics in different two-phase systems. Enzyme Microb Technol 5:352–360

Subramanian V (1986) Oxidation of propene and 1-butene by *Methylococcus capsulatus* and *Methylosinus trichosporium*. J Ind Microbiol 1:119–127

Ward OP, Young CS (1990) Reductive biotransformations of organic compounds by cells or enzymes of yeast. Enzyme Microb Technol 12:483–493

Weijers CAGM, Bont JAM de (1991) Enantioselective degradation of 1,2-epoxyalkanes by *Nocardia* H8. Enzyme Microb Technol 13:306–308

Weijers CAGM, Ginkel CG van, Bont JAM de (1988a) Enantiomeric composition of lower epoxyalkanes produced by methane-, alkane- and alkene-utilizing bacteria. Enzyme Microb Technol 10:214–218

Weijers CAGM, Haan A de, Bont JAM de (1988b) Chiral resolution of 2,3-epoxyalkanes by *Xanthobacter* Py2. Appl Microbiol Biotechnol 27:337–340