Regio- and Enantio-selectivity of the Cytosolic Epoxide Hydrolase-catalysed Hydrolysis of Racemic Monosubstituted Alkyloxiranes

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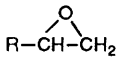
The rabbit liver cytosolic epoxide hydrolase-catalysed hydrolysis of racemic octyloxirane **1a**, butyloxirane **1b**, neopentyloxirane **1c** and t-butyloxirane **1d** to the corresponding ethane-1,2-diol derivatives was investigated and the regio- and enantio-selectivity of this reaction was determined for the first time. Attack by water always occurred at the primary carbon, with an (*R*) substrate enantioselection that was highly dependent on the branching of the alkyl chain and was the highest for compound **1d**. The results show a striking analogy with those found with the microsomal enzyme and suggest similar mechanisms and chiral discrimination abilities for the two epoxide hydrolases.

Epoxides are the main primary products of the Cytochrome P-450-dependent monooxygenase-catalysed oxidation of olefinic and aromatic compounds in mammals.[1] They can cause toxic, mutagenic and carcinogenic effects,[2,3] and can be detoxified enzymatically by the action of glutathione transferases[4] and microsomal and cytosolic epoxide hydrolases (mEH and cEH).[5,6] In contrast with the mEH-catalysed reactions, which have been widely investigated,[7] regio- and enantio-selectivity data are so far completely lacking for the cEH-promoted hydrolyses of racemic epoxides.† This information can be very useful in the evaluation of the efficiency of detoxification processes, as well as helping to throw light on the catalytic mechanism and the topology of the active site of the enzyme.[9,10] Although mEH and cEH are commonly considered to be endowed with distinct and complementary substrate specificities,[11] we found that both microsomal and cytosolic preparations easily hydrolysed monosubstituted alkyloxiranes. We therefore extended to cEH our recent investigation of the mEH-catalysed hydrolysis of these substrates.[1,2] The results show that the cytosolic enzyme exhibits regio- and enantio-selectivity towards racemic monosubstituted alkyloxiranes similar to that found for mEH.



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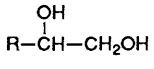

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R = **a**; n-C_8H_{17}
b; n-C_4H_9
c; CH_2CMe_3
d; Bu^t

Results and Discussion

Four racemic monosubstituted alkyloxiranes of different substituent chain length and branching, compounds **1a–d**, were subjected to hydrolysis with a rabbit liver cytosolic preparation, containing *ca.* 10 mg cm^{-3} of protein, at 37 °C and pH 7.4. Incubations were stopped by addition of methanol and extraction with ethyl acetate and the amounts of formed diols, **2a–d**, in the extracts were determined by GLC analysis. All four oxiranes were hydrolysed at initial rates that increased with increasing substrate concentration up to saturation of the solutions (~50 mmol dm^{-3}), so that enzyme saturation of the solution was never attained. Although the individual values exhibited fluctuations upon using different cytosolic preparations, the initial rates for saturated solutions of the linear-chain oxiranes **1a** and **1b** were always similar (typically, *ca.* 80 nmol min^{-1} per mg protein), and those for the branched epoxides **1c** and **1d** were respectively about one-half and one-third, when the same cytosolic preparation was used.

The regioselectivity of the enzymatic hydrolysis was determined in the case of one linear, **1b**, and one branched epoxide, **1c**, by using a lyophilized cytosolic preparation re-dissolved in 50% $H_2{}^{18}O$ to a final protein concentration of 15 mg cm^{-3} and 50 mmol dm^{-3} epoxides. Diols **2b** and **2c** obtained after complete hydrolysis were analysed by GLC-MS. The ratio of the peaks at *m/z* 61 and 63 (M − R)[14] showed a 50% content of ^{18}O, 97% of which resulted from incorporation at C(1) of compounds **2b** and **2c** on the basis of the peaks at *m/z* 87 and 89, and at 101 and 103 (M − CH_2OH), respectively. Therefore cEH, like mEH,[12,15] catalyses attack by water at the least substituted oxirane carbon, and this behaviour can be reasonably assumed to be general for the enzymatic hydrolysis of monosubstituted oxiranes.

In order to determine the absolute configuration and the enantiomeric excess (ee) of diols obtained on partial hydrolysis of the racemic epoxides, incubations were stopped at different times, and the formed diols, after quantification by GLC, were purified by column chromatography for measurement of their optical rotation. No rotation was found for compound **2a** even at very low substrate conversion. Diols **2b** and **2c** were instead dextrorotatory and diol **2d** was laevorotatory, the absolute values of the optical rotation obtained at comparable conversions being, however, dependent on the oxirane concentration in the incubation mixtures. The relation between optical rotation and absolute configuration was known for diols (*R*)-(+)-**2b**,[16] (*R*)-(+)-**2c**[12] and (*R*)(−)-**2d**.[17] Since attack by water occurred at C(1), the reactions involved retention of configuration at the chiral oxirane carbon. Thus, the formation of an excess of (*R*) diols was the consequence of a preferential hydrolysis of the (*R*) enantiomers of the oxiranes.

The ee of the diols, produced at several substrate conversions, was determined by HPLC analysis of their diastereoisomeric bis[MTPA] esters, obtained by reaction with (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride.‡ The results are reported in Table 1.

† The only stereochemical data so far reported for cEH reactions concern the product enantioselectivity of the hydrolysis of *meso*-epoxides.[8]

‡ Systematic name: β,β,β-trifluoro-α-methoxy-α-phenylpropionyl chloride.

Table 1 Enantiomeric excesses of diols obtained by cEH-catalysed hydrolysis of oxiranes **1a–d**

Substrate	Concentration of substrates (mmol dm^{-3})	Yield of diol[a] (%)	ee (%)	Absolute configuration
1a	25	5	0	
	sat.[b]	13	0	
1b	25	8	0	
	sat.[b]	12	24	*R*
	sat.[b]	45	24	*R*
1c	25	5	12	*R*
	sat.[b]	13	46	*R*
1d	25	17	44	*R*
	sat.[b]	13	100	*R*
	sat.[b]	45	100	*R*

[a] Complete hydrolysis was always obtained within several hours for 50 mmol dm^{-3} substrates. [b] Saturated: incubation mixtures contained *ca.* 0.1 mmol cm^{-3} of substrate.

The data in Table 1 show a very marked effect of chain branching on the enantioselectivity of the cEH-catalysed hydrolyses. n-Alkyl groups, as butyl or octyl, caused reactions showing very low or no selectivity, while branched alkyl groups induced substrate selection in favour of the (*R*) enantiomer of the oxiranes. Under comparable conditions, this enantioselectivity decreased with increasing distance of the branching from the oxirane ring, and raised with increasing substrate concentration. In epoxide-saturated solution only the (*R*) enantiomer of t-butyloxirane, **1d**, was hydrolysed by cEH during the first half-reaction.

These results, and particularly the order of increasing (*R*) enantiomer selection, show a striking analogy with those found for the rabbit liver mEH-catalysed hydrolysis of the same racemic substrates.[1,2] The possibility that the EH activity of the employed cytosolic preparations was actually due to contamination by the microsomal enzyme was excluded, since the microsomal and cytosolic fractions from the same preparation exhibited comparable activities towards the currently investigated monosubstituted alkyloxiranes, but very different activities towards other epoxides.[8] In conclusion, while more comparative data are highly desirable, the present results appear to support the previous suggestion, based on the product enantioselectivity of the mEH- and cEH-catalysed hydrolysis of *meso*-1,2-epoxycycloalkanes and *cis*-stilbene oxide,[8] of similar mechanisms and chiral discrimination abilities of the active sites of the two enzymes in the rabbit.

Experimental

Materials.—Commercial racemic octyloxirane **1a** (Aldrich-Chemie; 99%), butyloxirane **1b**, t-butyloxirane **1d**, decane-1,2-diol **2a**, hexane-1,2-diol **2b** and 3,3-dimethylbutane-1,2-diol **2d** (all Aldrich-Chemie; >98%) were used after distillation. Neopentyloxirane **1c** and 4,4-dimethylpentane-1,2-diol **2c** were obtained as reported.[1,2]

Hepatic cytosolic preparations were obtained from male New Zealand white rabbits (2.5–3 kg) by the previously reported procedure[13] and stored at −40 °C. Samples (1 cm^3) were also immediately lyophilized and rehydrated before use by addition of 50% $H_2{}^{18}O$ (1 cm^3).

Initial-rate Measurements.—The experiments were carried out by addition of samples (40 mm^3) of 1–5 mol dm^{-3} ethanolic stock solutions of oxiranes **1a–d** to a cytosolic preparation (protein content 10 mg cm^{-3}; pH 7.4) (2 cm^3) followed by incubation at 37 °C for 30 min or for 3 h. The reactions were stopped by addition of methanol (2 cm^3) containing *trans*-cyclohexane-1,2-diol as a standard for the quantification of the formed diols, and the supernatants were analysed by GLC (2 m glass column, 2.5 mm id, packed with 10% NPGS on silanized Chromosorb 80–100 mesh: column 170 °C, nitrogen flow 60 cm^3 min^{-1}). Diol formation was always linear with time within the first 3 h. No oxirane hydrolysis was observed when boiled cytosolic preparations were used. The obtained initial hydrolysis rates for oxirane-saturated solutions are given in the text.

Regioselectivity of the Hydrolyses.—A 2.5 mol dm^{-3} solution of oxirane **1b** or **1c** (20 mm^3) was added to a 50% $H_2{}^{18}O$ cytosolic preparation (protein content 15 mg cm^{-3}) (1 cm^3). After incubation at 37 °C for 18 h, methanol (1 cm^3) was added and the mixtures were extracted with ethyl acetate. *trans*-Cyclohexane-1,2-diol was added to a sample of the extracts as a standard for GLC quantification of the formed diols. GLC analysis showed complete hydrolysis of both oxiranes. GLC-MS analyses were carried out at 70 eV.

Product Isolation.—Incubations were carried out as described above, using cytosolic preparation (protein content 10 mg cm^{-3}) (20 cm^3) at the oxirane concentrations reported in Table 1. At due times, the reactions were stopped by addition of methanol (5 cm^3) and the mixtures were extracted with ethyl acetate (3 × 15 cm^3). *trans*-Cyclohexane-1,2-diol was added to a sample of the extracts as a standard for GLC quantification of the formed diols. The remaining extracts were evaporated and the diols were isolated by column chromatography over silica gel [eluent (3:2) hexane–ethyl acetate] and the optical rotation was measured in methanolic solution.

Enantiomeric Excesses.—Diols **2a–d**, isolated from enzymatic hydrolyses, were converted into their bis[MTPA] esters,[1,2] which were analysed by HPLC (UV detector; normal-phase Hypersil column, 15 cm; 5 μm) under the following conditions: (98:2) hexane–ethyl acetate, 1.5 cm^3 min^{-1}, λ 260 nm, for the bis[MTPA] esters of diols **2a**, **2b** and **2c**; (99.7:0.3) hexane–acetone, 1.5 cm^3 min^{-1}, λ 240 nm, for the bis[MTPA] ester of diol **2d**. The enantiomeric excesses, obtained from the ratio of the peaks of the two diastereoisomeric bis[MTPA] esters, are reported in Table 1.

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