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Different Enantioselectivity and Regioselectivity of the Cytosolic and Microsomal Epoxide Hydrolase Catalyzed Hydrolysis of Simple Phenyl Substituted Epoxides.≠

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Abstract: The cytosolic (cEH) and the microsomal epoxide hydrolase (mEH) hydrolyse styrene oxide and *trans*-1-phenylpropene oxide with different enantioselectivity and regioselectivity. While mEH always leads to a regiospecific and enantioselective opening at the non-benzylic oxirane carbon, cEH gives a non-regioselective and non-enantioselective attack to styrene oxide and a regiospecific and non-enantioselective attack at the benzylic carbon of 1-phenylpropene oxide.

Epoxide hydrolases (EH) are important enzymes involved in the detoxification of the often mutagenic and carcinogenic epoxides arising from the Cytochrome P-450 catalyzed biooxidation of many xenobiotics.[1]

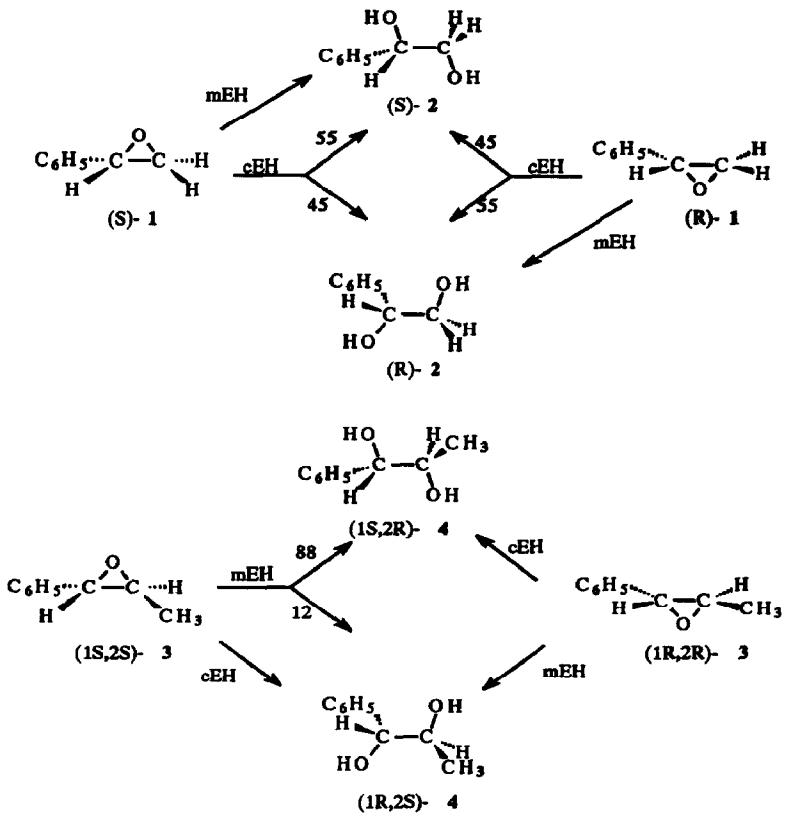
Two main EH forms, a microsomal (mEH)[2] and a cytosolic epoxide hydrolase (cEH),[3] both catalyzing an anti addition of water to the oxirane ring to give vicinal diols and having broad but different substrate selectivities, are known. The microsomal enzyme has been more extensively investigated from the stereochemical and mechanistic point of view. A remarkable regioselectivity[4] and substrate and product enantioselectivity[5-7] have been associated with mEH, but are less generally documented for cEH.[8-11] Until very recently, a direct, general base-catalyzed attack of water at one oxirane carbon has been commonly considered as the mechanism for the mEH reactions,[12] based on the results of ^{18}O-tracer studies,[4b] the lack of metal ions involvement,[4b] the absence of general acid catalysis,[13] and the essential role of a histidine residue.[14] Recent single turnover experiments conducted in $H_2{}^{18}O$ and with the enzyme covalently labelled with ^{18}O, as well as a limited but rather striking sequence similarity between mammalian mEH and bacterial haloalkane dehalogenases (HAD), have, however, shown[15] that the catalytic mechanism of mEH involves an ester intermediate (probably aspartate), which is subsequently hydrolysed with the assistance of a histidine residue.

Nothing is known at present about the catalytic mechanism of cEH, although comparative regioselectivity and enantioselectivity studies of the two epoxide hydrolases using aliphatic epoxides seemed to suggest similar mechanisms and steric requirements of the active sites of the two enzymes in the rabbit.[7,8]

In this communication we are reporting for the first time a markedly different regioselectivity of the rabbit liver mEH and cEH catalyzed hydrolyses of phenyl substituted epoxides, resulting in a different

≠Dedicated to Professor Giancarlo Berti on the occasion of his 70th birthday.

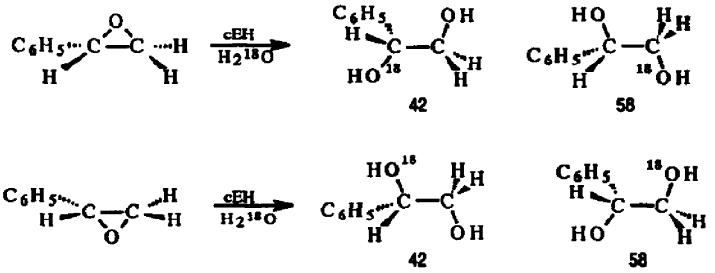
stereochemical outcome of these reactions with enantiomerically pure substrates. A complete lack of substrate enantioselectivity is also being reported for the cEH reactions of these epoxides, in contrast with the mEH reactions.



Rabbit liver microsomal preparations, containing mEH, catalyzed the hydrolysis of both (S)- and (R)-styrene oxide (1) to 1-phenylethane-1,2-diol (2) with complete retention of configuration by a >98% regiospecific attack at C(2).[16] A remarkable kinetic resolution, favouring the hydrolysis of the (R) enantiomer, was observed in the mEH reaction of (±)-1.[16] Likewise, a >98% regiospecific attack at C(2) was found for the rabbit liver mEH promoted hydrolysis of the (1R,2R) enantiomer of *trans*-1-phenylpropene oxide (3) to give (1R,2S)- 1-phenylpropane-1,2-diol (4), while an only 88% regioselective attack at the same C(2) occurred in the case of (1S,2S)-3.[16] A modest enantioselection in favour of the (1S,2S) enantiomer was found in the hydrolysis of (±)-3.[16]

Both enantiomers of styrene oxide and of *trans*-1-phenylpropene oxide (5-30 mM) were hydrolysed to diols 2 and 4 when incubated at 37 °C and pH 7.4 with a crude cytosolic preparation, obtained from rabbit livers[17] and diluted to a protein concentration of 3-6 mg/ml. The diols were quantified by HPLC as reported.[16] The saturation rates of diol production were respectively: (S)-1, 8; (R)-1, 6; (1R,2R)-3, 39; (1S,2S)-3, 41 nmol/(mg x min). When racemic styrene oxide (10 mM) was incubated with a cytosolic

preparation containing 12 mg protein/ml and the reaction was stopped at several conversions between 10 and 50% by extraction of the unreacted substrate and of the formed diol with ethyl acetate, GLC on a Chiraldex G-TA column, after acetylation of the diol,[16] showed that practically racemic 1 and 2 were always recovered. Nearly racemic 1-phenylethane-1,2-diol (R/S = 45:55 from (S)-1, R/S = 55:45 from (R)-1) was likewise shown by GLC to be produced when both (R)- and (S)-1 were incubated under similar conditions. The slow nonenzymatic hydrolysis of (R)- and (S)-1 in a Tris-HCl buffer, pH 7.4, at 37 °C occurred with 73% inversion of configuration at C(1) and gave a < 3% contribution to the cEH catalyzed reaction. The possibility that the stereochemical outcome of the enzymatic hydrolysis of (R)- and (S)-1 was due to a dehydrogenation of a first formed diol 2 of retained configuration to hydroxymethyl phenyl ketone followed by reduction back to 2 by the action of enzymes eventually present in the crude cytosolic preparation was safely excluded, since no racemization was observed when the optically pure 1-phenylethane-1,2-diols were incubated in the cytosolic preparation under conditions identical to those used for the hydrolysis of 1. In principle, two possibilities could account for the observed stereochemistry of the cEH catalyzed hydrolysis of the two enantiomers of styrene oxide. First, a regiospecific but not stereoselective water attack involving a competitive anti and syn opening at C(1). Second, a non-regioselective attack at C(1) and C(2), the former occurring with inversion and the latter with retention of configuration at C(1). The second hypothesis was proved to be the correct one by carrying out the hydrolysis of both enantiomers of 1 with a lyophilised cytosolic preparation[8] redissolved in 98% $H_2{}^{18}O$ to a final protein content of 12 mg/ml, followed by GLC-MS analysis of the produced diols 2. The ratio of the M+2 and M peaks showed that ≥ 97% ^{18}O was incorporated in the diol, while that of the 107 and 109 peaks, due to $C_6H_5CH^{16}OH^+$ and $C_6H_5CH^{18}OH^+$ respectively arising from the loss of $CH_2{}^{18}OH^+$ and $CH_2{}^{16}OH^+$ from the molecular ion, was 1.4 ± 0.1. Thus, at variance with the mEH catalyzed reaction, the cEH promoted hydrolysis of styrene oxide is lacking of regioselectivity and substrate enantioselectivity.



GLC analysis on the same chiral column, carried out after acetylation of diol 4,[16] showed that racemic 1-phenylpropane-1,2-diol and racemic *trans*-1-phenylpropene oxide were recovered from incubations of (±)-3 with the same cytosolic preparation under the conditions employed for the hydrolysis of 1, stopped at several conversions between 10 and 50%. On the other hand, GLC analysis revealed the formation of the (1R,2S)-(-)-enantiomer of 1-phenylpropane-1,2-diol (4) from (1S,2S)-(-)-3 and of (1S,2R)-(+)-4 from (1R,2R)-(+)-3 in ≥98% enantiomeric excesses. The configurations of these diols have been identified by the GLC retention times on the chiral column, and by the measurement of the optical rotations[18] after isolation of the products by column chromatography. Thus, also in this case the reaction did not exhibit any substrate

enantioselectivity, but involved an anti stereospecific and regiospecific attack at C(1), i.e. a regiochemistry opposite to that of the mEH reactions of the same substrates **3**.

It must be stressed that these are the first reported examples of EH-catalyzed opening of simple monosubstituted epoxides in which nucleophilic attack occurs at both oxirane carbons, and of 1,2-disubstituted epoxides in which ring opening takes place exclusively at the carbon bearing the larger substituent. This behaviour appears to be peculiar to the cEH reactions of phenyl substituted epoxides, and suggests that epoxide protonation, favouring opening at the benzylic carbon, is more important for cEH than for mEH. The use of substrates appropriately substituted on the phenyl ring may help to check this hypothesis.

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