Lipase-mediated Formation of Peroxycarboxylic Acids used in Catalytic Epoxidation of Alkenes

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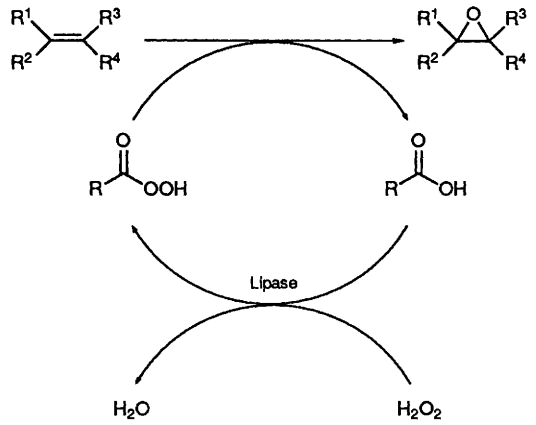
Epoxidation of alkenes was achieved under extremely mild conditions by employing peroxycarboxylic acids formed continuously *in situ* by lipase-catalysed perhydrolysis of the corresponding carboxylic acids.

During recent years the use of lipases in organic chemical processing has been studied extensively and the technologies for production and application of lipases have been highly developed. As a consequence, the lipases are now recognized as efficient and useful catalysts for modification of fats and oils by acidolysis of triglycerides and for synthesis or hydrolysis of carboxylic acid esters.[1] In these reactions the lipases often exhibit a high regio- and stereo-selectivity which may be exploited for synthesis of optically active compounds.[2,3] Furthermore the lipases offer unique benefits due to the mild reaction conditions employed in lipase-catalysed reactions.

The activity of lipases towards peroxy-compounds has not



R = Alkyl; R^1 = Alkyl; R^2,R^3,R^4 = H, Alkyl

Scheme 1

Table 1 Synthesis of peroxyoctanoic acid using various lipases[a]

Lipase derived from[b]	Peroctanoic acid formed in 120 min (mmol dm^{-3})[c]
Candida antarctica	33
Mucor miehei	0
Humicola sp.	19
Candida cylindracea	20
Pseudomonas sp.	25

[a] The standard reaction was performed at room temperature by adding 153 μl of 60% (w/v) hydrogen peroxide to a stirred suspension of 4500 BIU immobilised lipase (typically 20 mg) in 10 ml of a 100 mmol dm^{-3} solution of octanoic acid in hexane. 1 Batch interestirification unit (BIU) corresponds to 1 mol of hexadecanoic acid incorporated into triolein per minute (initial activity). [b] The lipase preparations used were as previously described.[3] [c] Peroxyoctanoic acid levels were monitored by HPLC using a Merck[R] LicroSorb RP-18 column, a gradient of MeCN–H_2O (0.1% HCO_2H) as mobile phase, and UV detection (210 nm). Peroxyacid reference was synthesized according to the literature.[8]

Table 2 Synthesis of various peroxycarboxylic acids in hexane using immobilized *C. antarctica* lipase[a]

Carboxylic acid	Peroxyacid formed in 120 min (mmol dm^{-3})
Octanoic acid	33
Decanoic acid	41
Dodecanoic acid	54
Tetradecanoic acid	35
Hexadecanoic acid	35

[a] Experimental conditions were as described in Table 1.

been a subject of much attention. So far, only the capability of certain lipases to catalyse perhydrolysis (lysis by hydrogen peroxide) of carboxylic acid esters, thereby forming peroxycarboxylic acids in aqueous hydrogen peroxide solutions,[4] and stereospecific lipase-catalysed synthesis of acetic acid esters of organic hydroperoxides have been reported.[5] The possibility of generating peroxycarboxylic acids directly from carboxylic acids with the aid of lipases has to our knowledge, not been investigated previously.

Table 3 Oxidation of various alkenes by peroxyoctanoic acid in hexane

	Yield of epoxide in 4 h (%)[a]	
Alkene	Catalytic oxidation[b]	Chemical oxidation[c]
Cyclohexene	2	10
3-Ethylpent-2-ene	48	50
Tetramethylethylene	99	74

[a] Yields were determined by capillary GC using a Supelcowax™ 10 column and flame ionisation detection. [b] The catalytic oxidation was carried out at room temperature by adding 25 μl of 60% (w/v) H_2O_2 (0.5 mmol) to a stirred suspension of 100 mg *C. antarctica* lipase in 10 ml of a solution of both the alkene and octanoic acid in hexane, 10 mmol dm^{-3} with respect to both. [c] The chemical oxidation was carried out simply by adding 10 mmol dm^{-3} preformed peroxyoctanoic acid (prepared according to the literature[8]) to 10 ml of a 10 mmol dm^{-3} solution of the alkene in hexane.

In continuation of our work on application of enzymes in organic synthesis, we have found that immobilised lipases can be applied for generating peroxycarboxylic acids in a suitable organic solvent directly from the parent carboxylic acid and hydrogen peroxide. Furthermore, the peroxy acids formed under these very mild reaction conditions can be applied concomitantly for epoxidation of alkenes. In this fashion epoxidation of alkenes can be carried out using carboxylic acids in catalytic amounts (Scheme 1).

Among several solvents tested for the lipase-catalysed peracid generation, the highest yields of peroxy acid were observed using toluene or hexane, while lower yields were achieved when using dioxane or acetonitrile. These results are in accordance with the observation that lipases generally perform better for synthetic purposes in water-immiscible rather than in water-miscible organic solvents.[6] The lipase-catalysed synthesis of peroxycarboxylic acid was thus advantageously performed in a two-phase system where the immobilised enzyme efficiently catalysed the reaction on the water–solvent interphase. Among the enzymes tested, an immobilised lipase from *Candida antarctica*[7] was found to be superior to other lipases with respect to synthesis of peroctanoic acid in hexane (Table 1). Furthermore, the *C. antarctia* lipase was found to be capable of generating a variety of peroxycarboxylic acids in hexane. The best yields were obtained in the case of medium-chain peroxycarboxylic acids which could be formed in concentrations of 54 mmol dm^{-3} (Table 2). The lipase was found to lose its activity completely by exposure to high hydrogen peroxide concentrations but to retain about 75% of its activity when treated with 50 mmol dm^{-3} peroctanoic acid for 20 h. Accordingly, yields of peroxycarboxylic acids could be increased by adding hydrogen peroxide gradually to the reaction mixture.

As indicated above, the smooth lipase-catalysed formation of peroxycarboxylic acids lends itself to lipase-catalysed synthesis of epoxides from alkenes and hydrogen peroxide in the presence of catalytic amounts of carboxylic acids (Scheme 1). The reaction can be performed simply by adding hydrogen peroxide as a 60% (w/v) solution to a suspension of the immobilised lipase in a solution of carboxylic acid and alkene in an organic solvent. By employing this procedure a smooth conversion of various alkenes to epoxides was achieved in yields almost identical to those obtained by chemical epoxidation using a preformed peroxycarboxylic acid (Table 3). In case of liquid alkenes the conversion was easily carried out simply by dispersing the immobilised lipase in the alkene and

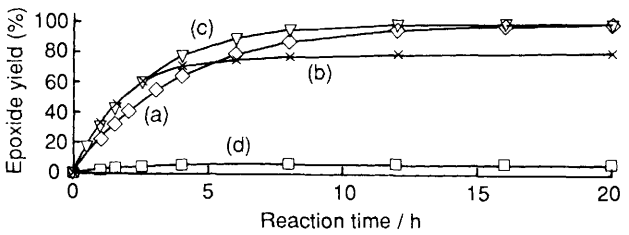


Fig. 1 Time course of solvent-free, lipase-catalysed epoxidation of various alkenes. [a] The reactions were performed by adding 235 μl of 60% (w/v) H_2O_2 at the beginning of the reaction and after 1, 1.5, 2.5, and 4 h respectively (a total of 20 mmol) to a stirred suspension of 175 mg of immobilized lipase derived from *C. antarctica* in 1.75 ml of the alkene (*ca.* 15 mmol) containing 270 μl of octanoic acid (1.7 μmol). Yields were determined using capillary GC as described in Table 3. In the case of 3-ethylpent-2-ene, full conversion was reached after *ca.* 10 h; however, several byproducts were formed during the reaction. (*a*) Cyclohexene; (*b*) 3-ethylpent-2-ene; (*c*) tetramethylethylene; (*d*) oct-1-ene

gradually adding hydrogen peroxide. Almost quantitative yields of epoxide were obtained when treating several substituted alkenes with hydrogen peroxide and the *C. antarctica* lipase for about 15 h at room temperature (Fig. 1).

In comparison to the highly acidic conditions usually applied for *in situ* generation of peroxycarboxylic acids[8] the present method provides a very mild and simple alternative. Moreover, the method for lipase-mediated epoxidation of alkenes represents a safe and cost-effective epoxidation amenable for large-scale organic chemical manufacture of even sensitive epoxides. Finally, lipase-catalysed synthesis of peroxycarboxylic acids may prove usable in any other oxidation involving the use of peroxycarboxylic acids in organic solvents.

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