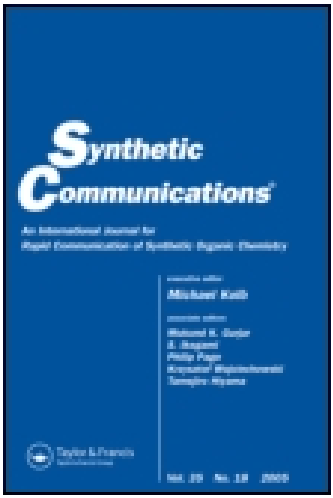

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Optimization of Chemo-Enzymatic Epoxidation of Cyclohexene Mediated by Lipases

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Abstract: This work describes the lipase-mediated epoxidation of cyclohexene. Lipases were used to generate peroxyoctanoic acid directly from octanoic acid and hydrogen peroxide and applied in situ to obtain cyclohexene oxide. Various parameters, which could affect this reaction, were studied such as lipases from different sources, organic solvents, temperature and acyl donor concentrations. Highest conversions to cyclohexene epoxide were achieved using a two-phase system of toluene or xylene/water with ROL (Amano F-Ap15 free *Rhizopus orizae* lipase) (84 and 80%) or CALB (Novozymes 435®-immobilized *Candida antarctica* lipase type B) (>9 and 84%) as biocatalysts. Using PSL (Amano PS-free *Pseudomonas* sp) the conversions were in the range of 12–53%, but an improvement was obtained by the use of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate (20 to 41% in water/methyl dichloride).

Keywords: Two-phase system, epoxidation, lipases

INTRODUCTION

The use of lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) as biocatalysts has been extensively explored in recent years and is still one of the most useful tools in the resolution of chiral compounds to obtain enantiopure building blocks in organic synthesis.[1,2] Although these reactions are usually carried out under "unnatural" conditions, it has appeared that the high degree of selectivity and efficacy exhibited by lipases in their natural

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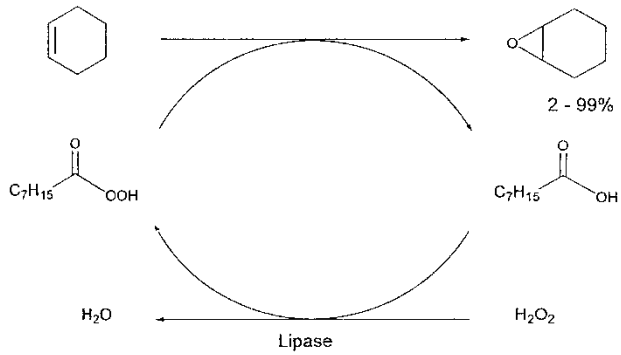
environment is also expressed in synthesis.[3,4] An example of these reactions is the lipase-catalyzed perhydrolysis of carboxylic acids and esters and the use of the resulting peroxyacids to form alkene oxides.[5,6]

Chemo-enzymatic epoxidation is a very useful reaction in organic synthesis. Skouridou et al. carried out the chemo enzymatic epoxidation of α-pinene at room temperature using *Candida antarctica* lipase as the biocatalyst (nearly 70% conversion).[7] Epoxides are key raw materials for a wide variety of products[8] and much effort has been devoted to the development of new active and selective epoxidation catalysts for processes that avoid the formation of large amounts of by-products.[9] One of the most used epoxidation processes is the stochiometric peracid route with meta-chloroperoxybenzoic acid (MCPBA),[10,11] but its commercial manufacture is under threat owing to its hazardous nature.[12] Also, this epoxidation method needs severe reaction conditions, for example, the use of a strong mineral acid, which is considered responsible for side reactions such as the formation of diols and hydroxyesters.[13]

Herein, the study of the experimental parameters, which affect the chemo-enzymatic epoxidation of cyclohexene, is reported, such as the use of lipases from different sources, temperature, organic solvents, and the effect of acyl donor concentrations. In this reaction, octanoic acid was converted by lipase-perhydrolysis to its respective peroxyoctanoic acid and then used in the epoxidation of cyclohexene in an aqueous/organic solvent biphasic system. For comparison, one reaction was also performed in a three-phase system of water/methyl dichloride/ionic liquid (Scheme 1).

RESULTS AND DISCUSSION

In our initial attempts, lipases from various sources were investigated to evaluate which of them could be used to produce cyclohexene oxide in



Scheme 1.

highest yields. Nine lipases, CALB (immobilized *C. antarctica* lipase type B immobilized in anionic resin, 10,000 PLU/g), ROL (free *Rhizopus orizae* lipase, 150,000 u/g), PSL (free *Pseudomonas* sp lipase, 30,000 u/g), MJL (free *Mucor javanicus* lipase, 10,000 u/g), CRL (free *Candida rugosa* lipase, 10,000 u/g), RML (free *Rhizomucor miehei* lipase, 1,000 u/g), TML (free *Thermomices lanuginosus*, 100,000 u/g), PPL (porcine pancreatic lipase, 135,000 u/g), and ANL (free *Aspergillus niger* lipase, 120,000 u/g) were selected as the enzymes. The conversions were determined by ^{1}H NMR. Table 1 shows the variation of the conversion degrees (in %) using different lipases.

These results demonstrated that the highest yields, 99%, were obtained when CALB was used in a biphasic system of water/toluene. This is in agreement with some literature data.[5,14] This suggests that this lipase is more resistant to the toxic media formed by the hydrogen peroxide and organic solvent. However, good results were also achieved when ROL and PSL were used in their native form, yielding 84% and 53% cyclohexene oxide, respectively. When lipases from MJL, CRL, and RML were employed, also in their native form, very low conversions to cyclohexene oxide were obtained. These were 12, 2, and <1%, respectively. TLL, PPL, and ANL were not appropriate for this conversion and no product was detected. Apparently, there is not a direct relation between the epoxide conversion degree and the enzyme activity.

The shape and the properties of binding sites for each lipase,[15] and the possible denaturation caused by hydrogen peroxide and the organic solvent, must also be considered. It is well known that a denaturation

Table 1. Chemo-enzymatic epoxidation of cyclohexene catalyzed by different lipases[a]

Lipase	Conversion (%)
CALB	>99
ROL	84
PSL	53
MJL	12
CRL	2
RML	<1
TML	n.d.[b]
PPL	n.d.[b]
ANL	n.d.[b]

[a]The reactions were performed with 5 mmol of cyclohexene, 10 mmol of octanoic acid, 1 mL of hydrogen peroxide 30%, 50 mg of each lipase, biphasic system: water/toluene at 30°C for 24 h.

[b]n.d. = not detected.

can occur because of a change in the microenvironment that surrounds these biocatalysts, which causes a change in their structural conformations and a loss of activity.[16,17]

The influence of temperature was also evaluated for the epoxidation of cyclohexene catalyzed by CALB for 24 h. In the range of 30–50°C, the corresponding epoxide was obtained in quantitative yields (>99%). Above 50°C, the thermostability of this enzyme was maintained;[18] however, the experimental conditions were not adequate, mainly because of hydrogen-peroxide decomposition and/or organic-solvent evaporation. Thus, a temperature of 30°C was selected for the following assays.

The influence of the organic solvent on the chemo-enzymatic epoxidation of cyclohexene was also studied. The three lipases chosen for these screenings were CALB, ROL, and PSL.

In most cases, the conversion in epoxide was dependent on the polarity of the organic solvent (expressed by log P values) and on the lipase source. However, there was not a linear relation with the solvent polarity.

Table 2 shows that the best yields were obtained in the water/toluene system regardless of the lipase source. This is in agreement with previous results.[14] High yields of cyclohexene oxide were also achieved when water/xylene was used, these being 53%, 80%, and 84% using PSL, ROL, and CALB, respectively. When chloroform was the organic solvent in the biphasic system, reasonable conversions in epoxide were also achieved. These were 50% and 65%, respectively, using PSL and CALB, and 37%

Table 2. Chemo-enzymatic epoxidation of cyclohexene in different biphasic systems[a]

		Lipase conversion (%)		
Organic solvent	log P	*Pseudomonas* sp	*Rhizopus orizae*	*Candida antarctica*
Ethyl ether	0.85	28	22	30
Methyl dichloride	0.93	20/41[b]	37	99
Chloroform	2.0	50	37	65
Toluene	2.5	53	84	99
Methyl tetrachloride	3.0	12	57	99
Xylene	3.1	40	80	84
Hexane	3.5	23	43	32
Heptane	4.0	21	42	44

[a]The reactions were performed with 5 mmol of cyclohexene, 10 mmol of octanoic acid, 1 mL of hydrogen peroxide 30%, 50 mg of each lipase, biphasic system: water/toluene at 30°C for 24 h.

[b]In a three-phase system of water/methyl dichloride/[Bmim][BF_4].

using ROL. Using CALB in methyl tetrachloride, hexane, and heptane, the conversions were 99%, 32%, and 44% respectively. When ROL was used in these solvents, the chemo-enzymatic epoxidation yielded considerable conversions. These were 57%, 43%, and 42%, respectively.

Using PSL, lower conversions in cyclohexene oxide were obtained. These were in the range of 12–53%. However, an improvement was obtained by the use of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4mim][BF_4]$) in a three-phase system of water/methyl dichloride/ionic liquid, increasing conversion from 20 to 41%. This result is also in agreement with those reported by Lau et al. for the transesterification of ethyl butanoate and ammoniolysis of ethyl octanoate.[19]

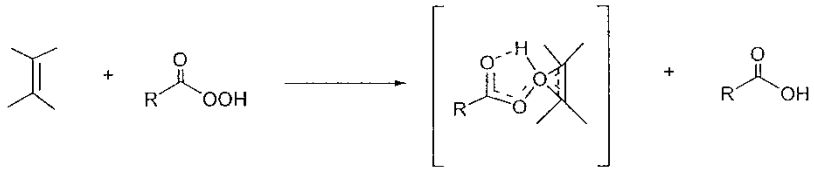
The systems studied here involve a biocatalytic perhydrolysis followed by a peroxyacid epoxidation. Thus, the solvent chosen must be suitable for the two reaction conditions and it is expected that its effect should be significant. In the first step the best organic solvents are those with a log P between 2.0 and 4.0, and in the second are those that can stabilize the transition state (**1**).[12] See Scheme 2.

The effect of octanoic acid (acyl donor) concentration on the process of epoxidation of cyclohexene was also investigated using CALB, ROL, and PSL (Figure 1).

As shown, the epoxide was obtained in quantitative yields using CALB and no dependence on the amount of acyl donor was observed in the range of 2.5–12.5 mmol. When ROL was the biocatalyst, the conversion in epoxide was in the range of 50–99%. The conversion was quantitative when 12.5 mmol of octanoic acid was the acyl donor. Reasonable conversions were obtained using PSL. These were in the range of 37–52%.

These data show that the conversion in the corresponding epoxide is more dependent on the nature of the lipase than the concentration of the acyl donor.

This study demonstrated that the lipase-mediated synthesis of cyclohexene oxide under mild conditions depends on various parameters such as the lipase source, organic solvent, temperature, and acyl-donor concentration. These results showed that these systems for enzyme-mediated epoxidation of alkenes in biphasic media are feasible. Further work is in progress in our laboratory to investigate other factors that can affect lipase stability. Also, more complex alkenes are currently under investigation.



(1)

Scheme 2.

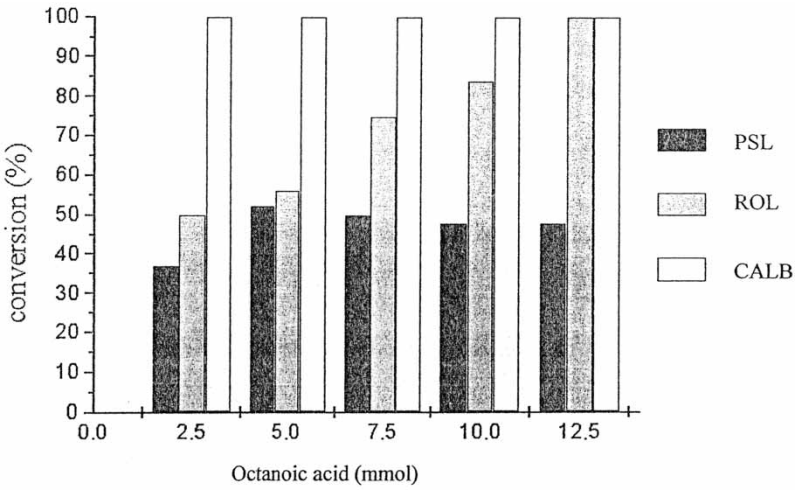


Figure 1. Degree of cyclohexene epoxidation as a function of octanoic acid concentration catalyzed by CALB, ROL, and PSL at 30°C for 24 h, H_2O_2 (30%, 1 mL), biphasic system: water/toluene.

EXPERIMENTAL

General

Commercial lipases from *C. antarctica* (Novozym 435®), *R. miehei* (Palatase M), and *T. lanuginosus* (Lipolase) were gently donated by Novozymes Latin America Ltda. (Brazil). Lipases from porcine pancreas (Amano PP), *C. rugosa* (Amano AY), *Pseudomonas* sp (Amano PS), *R. orizae* (Amano F-AP15), *M. javanicus* (Amano M), and *A. niger* (Amano A) were donated by Amano Pharmaceutical Co. (Japan). Ethyl ether was obtained from Dinâmica; methyl dichloride and chloroform from Nuclear; toluene from Merck; methyl tetrachloride, hydrogen peroxide 30%, and octanoic acid from Vetec; and xylene, heptane, and hexane from Grupo Química. Cyclohexene was obtained from Sigma/Aldrich Chemical Co. The ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4mim][BF_4]$) was prepared as described by Dupont et al.[20]

The ^1H NMR spectra were recorded in $CDCl_3$ (Aldrich) with TMS (Sigma) as the internal reference on a Bruker AC200F spectrometer. All reagents and solvents were analytical grade.

Chemo-emzymatic Epoxidation

In a typical experiment, distillated water (5 mL), an organic solvent (6 mL), hydrogen peroxide 30% (1 mL, 20 mmol), cyclohexene (5 mmol), lipases

from different sources (50 mg), and octanoic acid (2.5–12.5 mmol) were used. All reagents were added in a 125-mL, round-bottom flask, and this system was kept in a controlled temperature reactor (20–50°C) for 24 h. The yields of the crude product were evaluated by ^{1}H NMR by comparing the integral value of the olefinic hydrogen signal centered at 5.5 ppm with the oxirane-ring hydrogen signal centered at 3.1 ppm.

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