Lipase-mediated epoxidation of alkenes in supercritical carbon dioxide

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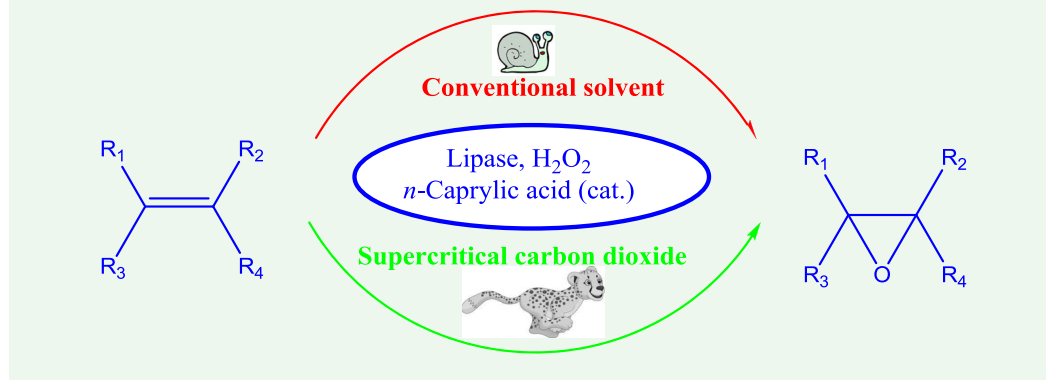
ABSTRACT

In this work, supercritical carbon dioxide was used as reaction media in the lipase-mediated epoxidation of alkenes for the first time. Under the optimal conditions (alkene (1 mmol), novozym 435 (15 mg), H_2O_2 (50% aqueous solution, 1.4 mmol), n-caprylic acid (0.05 mmol), 40°C, $scCO_2$ (10 MPa, 25 mL)), high yields (83–98%) of epoxidation of alkenes could be obtained in a short reaction time. Furthermore, novozym 435 exhibited a satisfactory reusability in the epoxidations. Thus, this work not only presents a green and mild method of lipase-mediated epoxidation with higher yield and reaction rate than other lipase-mediated routes ever reported but also expands the application of supercritical carbon dioxide in enzyme catalytic promiscuity.

Introduction

Epoxidation of alkenes is one of the important reactions in organic synthesis as the epoxy compounds are versatile building blocks in the manufacture of bulk and fine chemicals/intermediates (1). Some expensive and environmentally unacceptable processes such as organic peracids or metal catalysts/O_2 are still widely used in epoxidation reactions (2–5). At the forefront of existing challenges is the development of new mild oxidative methodologies that can be practical, efficient and minimize the environmental impact. Over the past few years, many reports have demonstrated that lipase could catalyze the *in situ* generation of peracids through a perhydrolysis of carboxylic acids or esters (6, 7). And the *in situ* formed peracids have been successfully utilized in many oxidations, such as epoxidation of

alkenes, Baeyer–Villiger reaction, Dakin oxidation, oxidations of amines, alcohols and sulfanyl compounds (8–15). These oxidative processes are representative examples of enzyme catalytic promiscuity, which is a term used to describe an enzyme that can catalyze chemical reactions fully different than physiological ones (16). Recent success in this significant field suggests new potential applications in enzyme-catalyzed organic synthesis (17, 18). Compared with the chemical methods, lipase-mediated oxidations benefit from the mild reaction conditions, environmental friendliness and green reusable catalyst. However, these methods have also encountered some drawbacks, such as long reaction time and the using of volatile organic solvents.
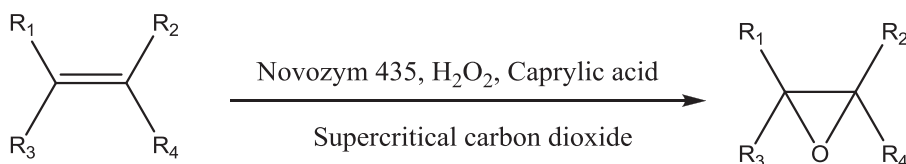
It is known that supercritical carbon dioxide ($scCO_2$) has a prodigious potential as an environmentally benign reaction medium for sustainable chemical

Scheme 1. Lipase-mediated epoxidation of alkenes in $scCO_2$

synthesis due to its advantages, such as low cost, non-toxicity, non-flammability, inertness, full recovery and moderate critical properties (Pc= 7.38 MPa, Tc = 304.2 K) when compared to other green solvents (*19–22*). Many researches showed that lipases are active and stable in $scCO_2$, which increased the potential use of $scCO_2$ in lipase-catalyzed reactions (*23–25*). For example, isoamyl acetate could successfully be synthesized from isoamyl alcohol in $scCO_2$ by novozym 435, and an esterification extent of 100% was obtained (*26*). Santos et al. reported that higher esterification rates could be obtained when the esterification of oleic acid with methanol was catalyzed by lipase in $scCO_2$ (*27*). These findings encourage further studies to develop $scCO_2$ as a reaction medium in enzymatic promiscuous reactions, such as lipase-mediated epoxidation.

As part of our program in environmentally friendly biocatalysis, we are interested in designing a "green" process to effectively oxidize a variety of alkenes. In this study, lipase-mediated epoxidation of alkenes in $scCO_2$ is reported for the first time (Scheme 1). Furthermore, this work also provides the first example of $scCO_2$ as reaction media in enzymatic promiscuous reactions, which broadens the application of $scCO_2$ in biocatalysis.

Results and discussion

According to the previous reports (*28, 29*), novozym 435 (a commercial immobilized *Candida antarctica* lipase B) has always been used to be the efficient catalyst combined with H_2O_2 as an oxidant for the lipase-mediated epoxidation, and novozym 435 exhibits a good stability when in these reports. Furthermore, the use of H_2O_2 as an oxidant for the lipase-catalyzed perhydrolysis is much more promising from economic and environmental points of view as the only byproduct (H_2O) is environmentally benign (*30*). Therefore, we selected H_2O_2 as the oxidant and novozym 435 as the catalyst for the perhydrolysis of *n*-caprylic acid to generate peracid.

In this study, a stainless-steel pressure-resistant vessel (internal volume: 25 mL) was used as the reactor. Initially, styrene was adopted as the model substrate for the epoxidation. Different reaction media were investigated and the results were shown in Table 1. The highest

yield ($92 \pm 1.2\%$) could be obtained when $scCO_2$ (10 MPa) was used as the reaction medium. To our knowledge, compared with other lipase-mediated epoxidation reported so far, novozym 435 presents the highest catalytic efficiency when $scCO_2$ was used as the reaction medium. The higher diffusivity and lower viscosity and surface tension of $scCO_2$ are responsible for the reduction of interphase transport limitations, thus increasing the reaction rate in this lipase-mediated epoxidation. And trace epoxidative product could be obtained in the control experiments (entry 5–6) which suggested that the active conformation of the enzyme was necessary for this enzymatic process. The above results were according to the literature which reported that hydrogen peroxide alone only presented very poor epoxidative ability in $scCO_2$ (*31*). Furthermore, we also examined the high-pressure *n*-hexane as the reaction medium on this epoxidation (entry 7), and the yield was almost the same with that under atmospheric pressure due to the unchanged properties of *n*-hexane under different pressures.

As a function of results in supercritical media, the effect of different $scCO_2$ conditions, determined by all the following temperatures (35, 40 and 45°C) and pressures (10–30 MPa), on the lipase-mediated epoxidation of styrene was investigated. As shown in Table 2, when the temperature was fixed, the yield of epoxidation decreased with the increasing of pressure from 10 to 30 MPa. The increase in pressure, which involves an increase of the $scCO_2$ density, produced a decline in the enzyme performance. And also, a higher pressure

Table 1. Lipase-mediated epoxidation of styrene in different solvents[a].

Entry	Enzyme	Solvent	Time (h)	Yield (%)
1	Novozym 435	*n*-Hexane	48	32 ± 2.8
2	Novozym 435	Acetonitrile	48	86 ± 1.6
3	Novozym 435	Ethyl acetate	1	21 ± 3.2[b]
4	Novozym 435	$scCO_2$ (10 MPa)	1	92 ± 1.2
5	None	$scCO_2$ (10 MPa)	1	Trace
6	Novozym 435[c]	$scCO_2$ (10 MPa)	1	Trace
7	Novozym 435	*n*-Hexane (10 MPa)	48	30 ± 2.4

[a]Reaction conditions: styrene (1 mmol), novozym 435 (15 mg), H_2O_2 (50% aqueous solution, 1.4 mmol), *n*-caprylic acid (0.05 mmol), 40°C, solvent (25 mL), 1 h.
[b]Ethyl acetate was used as the solvent and peracid precursor, and urea-hydrogen peroxide was used as oxidant. [c]Pretreated enzyme by heating it for 5 h at 100°C in water.
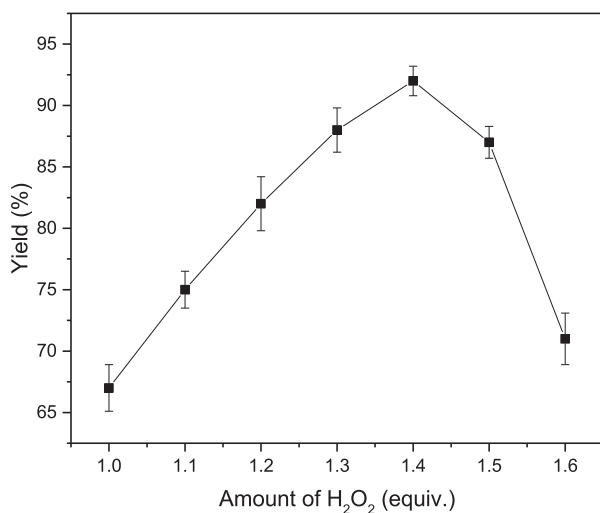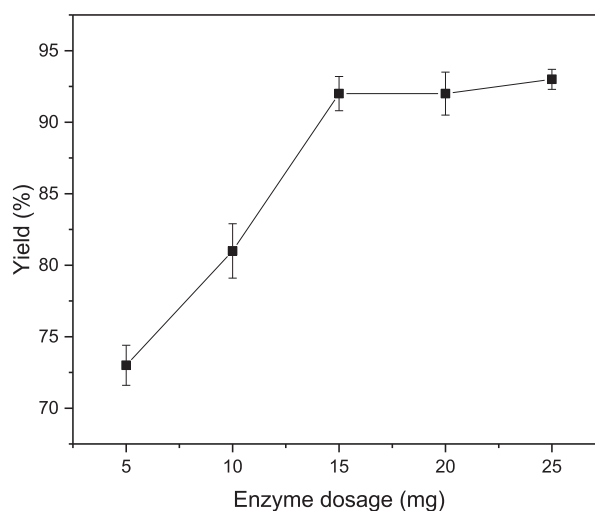
Table 2. Effect of temperature and pressure on lipase-mediated epoxidation in scCO$_2$ [a]

Entry	Temperature (°C)	Pressure (MPa)	Yield (%)
1	35	10	86 ± 0.9
2	35	20	83 ± 1.8
3	35	30	76 ± 1.5
4	40	10	92 ± 1.2
5	40	20	88 ± 1.6
6	40	30	81 ± 2.1
7	45	10	65 ± 2.5
8	45	20	62 ± 1.9
9	45	30	51 ± 1.1

[a]Reaction conditions: styrene (1mmol), enzyme dosage (15 mg), *n*-caprylic acid 0.05 mmol, H$_2$O$_2$ (1.4 mmol), 1 h.

could inactivate enzyme in an enzymatic reaction. The temperature obviously affected the lipase-mediated epoxidation at all the assayed pressures and the highest yield could be obtained at 40°C. High temperature could decrease in fluid density, which produced a reduction in internal diffusion limitations into the enzyme particle. However, higher temperature promoted the degradation of H$_2$O$_2$, such that the generation of peracid reduced dramatically.

Generally, the amount of oxidant might affect the yield of epoxidation. In this study, the effect of the amount of H$_2$O$_2$ was investigated (Figure 1). The yield of expoxidation increased when the amount of H$_2$O$_2$ was increased from 1 to 1.4 equiv. After that, the yield was decreased markedly. High oxidant loading in scCO$_2$ induced the inactivation of lipase, and then the yield of epoxidation declined. Furthermore, higher dosage of H$_2$O$_2$ solution improved the water content of the reaction system, and made the enzyme conformation too flexible, which may also decrease the activity

Figure 2. Effect of enzyme dosage on lipase-mediated epoxidation in scCO$_2$ Reaction conditions: styrene (1 mmol), novozym 435, H$_2$O$_2$ (50% aqueous solution, 1.4 mmol), *n*-caprylic acid (0.05 mmol), 40°C, scCO$_2$ (10 MPa, 25 mL), 1 h.

of the enzyme. Therefore, 1.4 equiv. of H$_2$O$_2$ was selected for this lipase-mediated epoxidation in scCO$_2$.

The epoxidation of styrene was carried out in scCO$_2$ with different amounts of novozym 435 and the results were demonstrated in Figure 2. The temperature and pressure were fixed at 40°C and 10 MPa, respectively. It could be observed that a clear increase in the yield of epoxidation with an increasing enzyme amount from 5 to 15 mg, and similar yields were obtained when the enzyme amount was higher than 15 mg. Therefore, 15 mg of novozym 435 was sufficient for this reaction.

The main advantage of immobilized lipases over the free form is that the enzyme can be reused many times in the process, thus reducing the cost of catalyst (*32*, *33*). Thus, the reusability of novozym 435 was investigated to assess the economic potential in this epoxidation (Figure 3). The lipase was washed with *n*-hexane to remove styrene and epoxide after each cycle. The catalytic performance was retained in first three cycles. The yields of epoxides decreased to 82% after seven cycles. The gradual decrease of epoxidative yield might be attributed to the leakage of the enzyme from the support during the epoxidation. Besides, we scaled up the reaction system in a pressure-resistant vessel with internal volume of 1 L (styrene (40 mmol), novozym 435 (0.6 g), H$_2$O$_2$ (50% aqueous solution, 56 mmol), *n*-caprylic acid (2 mmol), scCO$_2$ (10 MPa), 40°C. The yield of epoxide was up to 95% after 1 h. It is noteworthy that the hydrolysis of styrene oxide was not observed in this lipase-mediated epoxidation. These results indicate that this green method has a high potential for practical application.

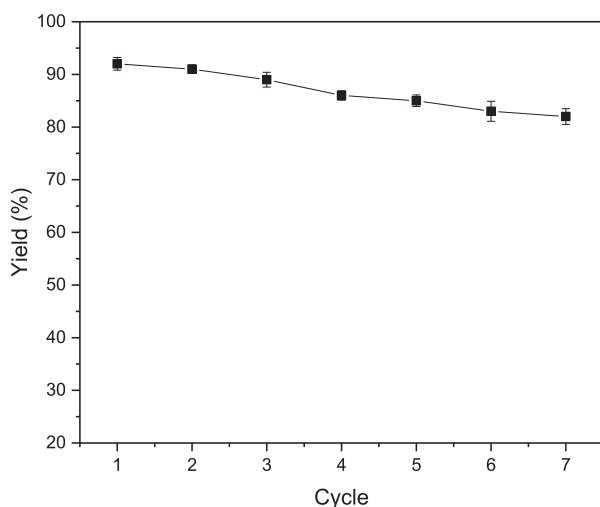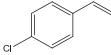**Figure 1.** Effect of amount of H$_2$O$_2$ on lipase-mediated epoxidation in scCO$_2$. Reaction conditions: styrene (1 mmol), novozym 435 (15 mg), H$_2$O$_2$ (50% aqueous solution), *n*-caprylic acid (0.05 mmol), 40°C, scCO$_2$ (10 MPa, 25 mL), 1 h.
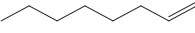
Figure 3. Reusability of novozym 435 on lipase-mediated epoxidation in scCO$_2$. Reaction conditions: styrene (1 mmol), novozym 435 (15 mg), H$_2$O$_2$ (50% aqueous solution, 1.4 mmol), n-caprylic acid (0.05 mmol), 40°C, scCO$_2$ (10 MPa, 25 mL), 1 h.

This new system was used for the epoxidation of other alkenes. As shown in Table 3, oxidation of aromatic alkenes gave the satisfactory yields of epoxides (entry 1–4). And the epoxidations of aliphatic alkenes (entry 5–6) were faster than those of aromatic alkenes, producing the corresponding oxides in 96–98% after 0.5 h. This result is in accordance with the previous reports (6–8).

Experimental materials

Novozym 435 was purchased from Sigma (Beijing, China). Silica gel was purchased from Qing Dao Hai Yang Chemical Industry Co. (Qingdao, China). The alkenes were of analytical reagent grade and purchased from J&K

Table 3. Lipase-mediated epoxidation of alkenes in scCO$_2^a$.

Entry	Alkene	Time (h)	Yield (%)
1		1	92 ± 1.2
2		1	83 ± 1.7
3		1	95 ± 0.7
4		1.5	85 ± 1.6
5		0.5	96 ± 0.6
6		0.5	98 ± 0.5

^{a}Reaction conditions: alkene (1 mmol), novozym 435 (15 mg), H$_2$O$_2$ (50% aqueous solution, 1.4 mmol), n-caprylic acid (0.05 mmol), 40°C, scCO$_2$ (10 MPa, 25 mL).

Scientific Ltd. (Beijing, China). All the other reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All the commercially available reagents and solvents were used without further purification. NMR spectra were taken with an Inova 500 (500 MHz) spectrometer. Carbon dioxide with a purity of 99.99% was from Changchun Juyang Gas Co., Ltd. The pressure in the system was controlled by a liquid CO$_2$ pump (Spe-ed SFE, Applied Separations, Inc.).

General procedure for the epoxidation of alkenes

Alkene (1 mmol), H$_2$O$_2$ (50% aqueous solution, 1.4 mmol), n-caprylic acid (0.05 mmol) and novozym 435 (15 mg) were mixed and sealed in a batch reactor (high-pressure-resistant stainless-steel vessel, 25 mL). The reaction was conducted at 10 MPa and 40°C. CO$_2$ gas was sent into the vessel by a CO$_2$ pump. The vessel was stirred with a magnetic stirrer. After the reaction to the desired time, depressurization and elution by n-hexane (10 mL*3) was conducted. The combined organic phases were concentrated under vacuum, and the resulting residue was purified by flash column chromatography on silica gel with EA/hexane (1/4). The products were analyzed by ^{1}H-NMR.

Conclusions

In summary, scCO$_2$ was reported to be superior to other conventional organic solvents for the lipase catalyzing the epoxidation of alkenes for the first time. In comparison with other lipase-mediated oxidation systems, this new oxidation system mediated by lipase in scCO$_2$ is greener and more efficient. Moreover, it is the first example for the application of scCO$_2$ in enzyme catalytic promiscuity. With this interesting finding, on-going studies on scCO$_2$ as a green medium for other enzymatic promiscuous reactions continue in our laboratory.

Disclosure statement

No potential conflict of interest was reported by the authors.

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