Efficient epoxidation of alkenes with hydrogen peroxide, lactone, and lipase†

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A green and efficient oxidation system containing hydrogen peroxide, lactone, and lipase was developed for the epoxidation of alkenes. A variety of alkenes was oxidized with this system, giving 87–95% analytical yield of the corresponding epoxides. The epoxidation occurred *via* lipase-catalyzed formation of hydroxy peroxy acid from lactone, without release of any harmful short-chain acid and alcohol, and *in situ* chemical oxidation of alkenes. Both hydrophilic ε-caprolactone and hydrophobic δ-decanolactone were shown to be good substrates to produce hydroxy peracids and good reaction solvents, and the method is suitable for the oxidation in either single phase or two-liquid phase. In comparison with other lipase-mediated oxidation systems, the new oxidation system gave higher yield, higher efficiency, and higher enzyme stability.

Introduction

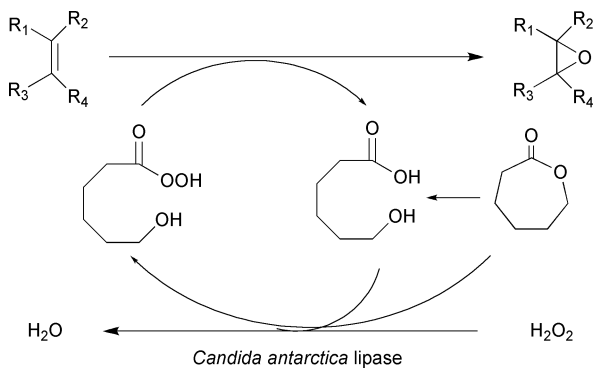
The development of green oxidations is very important for sustainable chemical industry. Many industrially useful oxidations such as Prileshajev-epoxidation, Baeyer–Villiger oxidation, and several metal-catalyzed oxidations are currently performed by using peroxycarboxylic acids.[1] While *m*-chloroperbenzoic acid is expensive and also hazardous, the other useful peroxy acids are highly unstable and dangerous to handle. *In situ* formation of the peroxy acids is thus necessary, and an enzymatic approach has received increasing attention due to green features. Lipase such as *Candida antarctica* lipase (Novozym 435®) is known to catalyze the perhydrolysis of carboxylic acid[2,3] and ester[2,4] with H_2O_2 to form peroxy acid. The *in situ* generated peroxy acid can perform the epoxidation of alkenes[2-5] or Baeyer–Villiger oxidation of ketones[6] in the same reaction system, giving rise to lipase-mediated oxidations. Since the perhydrolysis is reversible, the acid or ester has to be used in excess and in an organic solvent containing water as little as possible for efficient formation of peroxy acid. A more elegant approach is to use an ester such as ethyl acetate[7] or a carbonate such as dimethyl carbonate[7a] as the solvent as well as the perhydrolysis substrate, leading to successful epoxidation of several alkenes. However, such a system generated water-soluble short-chain acid and alcohol, such as acetic acid and ethanol in perhydrolysis of ethyl acetate, and methanol in the perhydrolysis of dimethyl carbonate, which inhibited the enzyme activity and reduced the enzyme stability.[8a] Moreover, the enzyme in the reaction system was also sensitive to high concentration of H_2O_2 and the short-chain peracid.[8b]

We are interested in developing a new oxidation system with lactone as substrate for lipase-catalyzed perhydrolysis and solvent for the chemical oxidation. No short-chain acid or alcohol could be generated during the formation of hydroxy peroxy acid from lactone and H_2O_2. In addition, different type of lactones such as hydrophilic ε-caprolactone and hydrophobic δ-decanolactone are easily available in large amounts, giving rise to different medium-chain hydroxy peracids as well as different systems in single or two-liquid phase for desired applications. Here we report our success on the development of a green, efficient, and stable oxidation system containing H_2O_2, lactone, and lipase and the *in situ* epoxidation of alkenes with this system.

Results and discussions

Lipase-mediated epoxidation of styrene with hydrogen peroxide in ε-caprolactone

The proposed reaction mechanism for the epoxidation with the new oxidation system is shown in Scheme 1, with ε-caprolactone as a representative of lactones. To prove the



Scheme 1 Lipase-mediated epoxidation of alkenes with hydrogen peroxide in ε-caprolactone.

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† Electronic supplementary information (ESI) available: GC chromatogram of epoxidation of olefins with hydrogen peroxide, ε-caprolactone and lipase. See DOI: 10.1039/b913077b

Table 1 Lipase-meditated epoxidation of styrene with hydrogen peroxide in ε-caprolactone

Entry[a]	K_2HPO_4 (mmol)	Novozym 435® (mg)	Conv. of styrene (%)[b]	Epoxide formed (%)[b]
1	0	50	86	45
2	1	50	88	76
3	1	20	85	76
4	1	10	79	73
5	0.5	20	92	84
6	0.3	20	91	84

[a] A mixture of styrene (1 mmol), H_2O_2 (50% aqueous solution, 2.5 mmol), Novozym 435® (10–50 mg, specific activity: 10 PLU/mg) and K_2HPO_4 (0–1 mmol) in ε-caprolactone (11 mmol, 1.5 mL) was shaken at 300 rpm and 30 °C for 24 h. [b] Concentrations of styrene and formation of styrene oxide were determined by GC analysis.

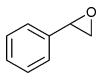
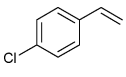
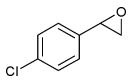
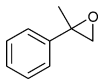
concept, the epoxidation of styrene was chosen as a model reaction. A mixture of styrene (1 mmol), H_2O_2 (2.5 mmol, 50% in aqueous solution), Novozym 435® (50 mg), and ε-caprolactone (1.5 mL) was shaken at 300 rpm and 30 °C for 24 h, converting 86% conversion of styrene and giving 45% of styrene oxide determined by GC analysis (Table 1, entry 1). This clearly demonstrated the feasibility of this novel system for epoxidation. The formation of styrene oxide is lower than the conversion of styrene, which was possibly caused by acid-catalyzed hydrolysis of styrene oxide similar to other lipase-mediated epoxidation systems. K_2HPO_4 was added to neutralize the acid in following experiments, and the formation of styrene oxide was increased to 76% (Table 1, entry 2). Further investigation was focused on the amount of enzyme as well as the amount of base. The best result was achieved with 84% formation of the epoxide by using 20 mg Novozym 435® and 0.3–0.5 mmol K_2HPO_4 (Table 1, entry 5–6).

Lipase-mediated epoxidation of different olefins with hydrogen peroxide in ε-caprolactone

This new system was used for the epoxidation of other alkenes. Oxidation of aromatic alkenes was represented with substituted styrenes, all giving excellent conversion to epoxides (Table 2, entry 1–3). Among them, α-methylstyrene was the best substrate giving 93% of α-methylstyrene oxide at 24 h (entry 3); epoxidation of 4-chlorostyrene was slower, giving 75% of 4-chlorostyrene epoxide after 48 h (entry 2). Nevertheless, 87% of this epoxide could be produced by using 40 mg Novozym 435® (entry 2). Remarkably, epoxidations of cyclic aliphatic alkenes such as cyclohexene and α-pinene are very fast, producing cyclohexene oxide and α-pinene oxide in 93–95% after 6–8.5 h (Table 2, entry 4–5). A typical time-course of the epoxidation of cyclohexene and α-pinene is shown in Fig. 1. Both epoxides were obtained in higher than 600 mM within 6–8.5 h, which implied the potential of practical application of this method.

The new oxidation system is convenient and efficient. In comparison with other systems using aqueous H_2O_2 in ethyl acetate or dimethyl carbonate,[7a] our system allowed for the addition of H_2O_2 in one portion instead of step-wise addition of H_2O_2 for 6 h.[7a] Moreover, it used 90% less enzyme, 85% less solvent and 50% less H_2O_2, but gave 10% higher yield in comparison with the epoxidation of styrene, α-pinene, and

Table 2 Lipase-mediated epoxidation of alkenes with hydrogen peroxide in ε-caprolactone

Entry[a]	Alkene	Epoxide	Time (h)	Epoxide formed (%)[b]
1			32	89
2			48	75 (87[c])
3			24	93[d]
4			8.5	95
5			6	93

[a] A mixture of alkene (1 mmol), H_2O_2 (50% aqueous solution, 2.5 mmol), Novozym 435® (20 mg, specific acitivity: 10 PLU/mg), K_2HPO_4 (0.5 mmol) and ε-caprolactone (11 mmol, 1.5 mL) was shaken at 300 rpm and 30 °C. [b] The formation of epoxide was determined by GC. [c] 40 mg Novozym 435®. [d] 4.5 mL ε-caprolactone.

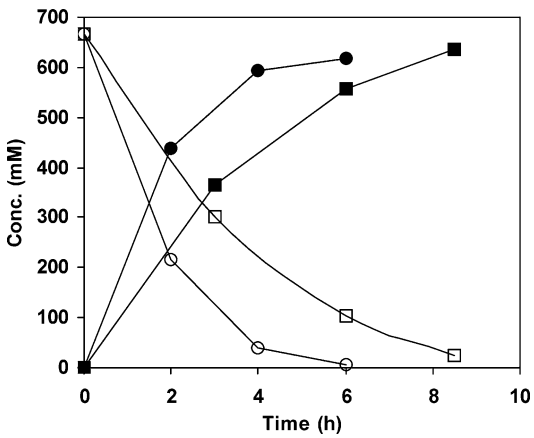


Fig. 1 Time-course of lipase-mediated epoxidation of cyclohexene and α-pinene with hydrogen peroxide in ε-caprolactone. Symbols: cyclohexene (□), cyclohexene oxide (■), α-pinene (○) and α-pinene oxide (●). Reaction conditions: see footnote *a* of Table 2.

cyclohexene in dimethyl carbonate and 20–30% higher yield in comparison with the epoxidation of α-pinene and cyclohexene in ethyl acetate.[7a] In comparison with the system using urea hydrogen peroxide (UHP) in ethyl acetate,[7b] similar yields and rates were achieved for the epoxidation of styrene and α-pinene, and higher yields and much faster rates were obtained for the epoxidation of cyclohexene and α-methylstyrene in our system using 60% less enzyme. Moreover, aqueous H_2O_2 is cheap and much greener than UHP due to higher atom efficiency and the production of water as a byproduct.

Reaction mechanism of lipase-mediated epoxidation with hydrogen peroxide in ε-caprolactone

To obtain understanding on the new oxidation system, the hydrolysis and perhydrolysis of ε-caprolactone with Novozym 435® were examined. Treatment of ε-caprolactone (1.5 mL) with Novozym 435® (20 mg) in the presence of 50% H_2O_2 (2.5 mmol) which contains 4.7 mmol H_2O was compared with the same treatment in the presence of 4.7 mmol H_2O: 6-hydroxycaproic acid was formed at 170 mM and 335 mM at 1 h in the former and latter case, respectively. These results clearly suggest the occurrence of hydrolysis of ε-caprolactone in the lipase-mediated epoxidation system (Scheme 1). The amount of 6-hydroxycaproic acid obtained with aqueous H_2O_2 was about the half of that produced with the same amount of H_2O, indicating the competition between the enzymatic hydrolysis with H_2O and the enzymatic perhydrolysis with H_2O_2. Based on the molar ratio of H_2O_2/H_2O of 2.5/4.7, the perhydrolysis seemed to be preferred, which is similar to the reported preference of lipase for H_2O_2 relative to H_2O with other types of substrates.[9] The enzymatic perhydrolysis of ε-caprolactone was further examined by the epoxidation of cyclohexene (1 mmol) using UHP (2.5 mmol) and Novozym 435® (20 mg) in ε-caprolactone (1.5 mL). 154 mM cyclohexene oxide was obtained at 1 h, demonstrating the similar epoxidation efficiency of using UHP as H_2O_2. Such a system contains no water, thus there is no hydrolysis of ε-caprolactone. Therefore, the successful epoxidation evidenced the formation of the hydroxy peracid from ε-caprolactone by enzymatic perhydrolysis. The possibility of perhydrolysis of 6-hydroxycaproic acid to the hydroxy peracid was also confirmed by the epoxidation of cyclohexene with Novozym 435® and H_2O_2 in acetonitrile containing 0.2 mmol 6-hydroxycaproic acid and compared with the similar system containing 0.2 mmol ε-caprolactone. The epoxidation happened in both cases, and the activity in the former case is about 60 times higher than that in the latter case. Thus, as shown in Scheme 1, the initial steps for the lipase-mediated epoxidation with ε-caprolactone and aqueous H_2O_2 are the perhydrolysis of ε-caprolactone with H_2O_2 to form 6-hydroxypercaproic acid and the hydrolysis of ε-caprolactone with H_2O to form 6-hydroxycaproic acid; the generated 6-hydroxycaproic acid is further perhydrolysed with H_2O_2 to give 6-hydroxypercaproic acid; the epoxidation of alkenes is then performed with 6-hydroxypercaproic acid which was transformed back to 6-hydroxycaproic acid.

Lipase-mediated epoxidation of styrene with hydrgen peroxide in δ-decanolactone

To further explore the generality of the concept, δ-decanolactone containing a 6-membered ring was examined for the epoxidation of styrene. δ-Decanolactone is hydrophobic and water insoluble, thus representing a type of lactone different from ε-caprolactone. While the oxidation system with ε-caprolactone and aqueous H_2O_2 contains a single liquid phase, the system containing δ-decanolactone and aqueous H_2O_2 forms a two-liquid phase. Epoxidation of styrene (1 mmol) with δ-decanolactone (1.5 mL), H_2O_2 (2.5 mmol) and Novozym 435® (50 mg) in the presence of K_2HPO_4 (1 mmol) gave styrene oxide in 43% at 24 h and 88% at 48 h, respectively. The oxidation is slightly slower than that with ε-caprolactone. The stability of enzyme in such a system was investigated by performing the epoxidation reaction with the recycling and reuse of the enzyme 3 times. The epoxide conversion at cycle 3 was as high as that at the initial one, as shown in Fig. 2. Thus, the new oxidation system containing hydrogen peroxide in δ-decanolactone is stable and enzyme can be recycled for repeated use. In contrast, the enzyme in a similar two-liquid phase oxidation system containing ethyl acetate, H_2O_2 and Novozym 435® was unstable and totally lost the activity in the first recycling and reusing experiment (Fig. 2).

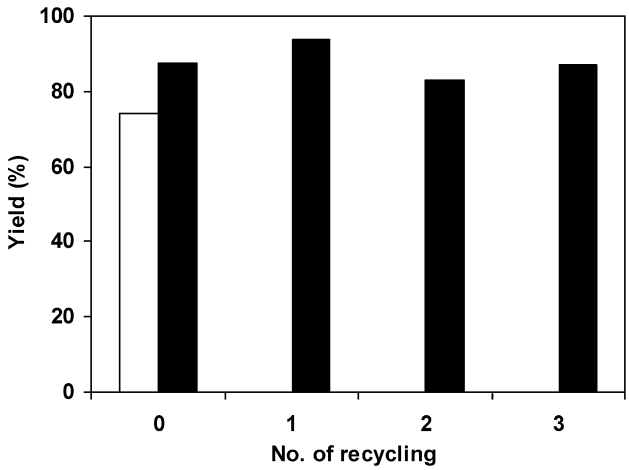


Fig. 2 Lipase-mediated epoxidation of styrene with H_2O_2 in δ-decanolactone (■) or ethyl acetate (□). Reaction conditions: a mixture of styrene (1 mmol), H_2O_2 (50% aqueous solution, 2.5 mmol), Novozym 435® (50 mg) and K_2HPO_4 (0.5 mmol) in δ-decanolactone (1.5 mL) or ethyl acetate was shaken at 300 rpm and 30 °C for 48 h. Enzyme recycling: enzyme was filtered after each batch of epoxidation, washed by hexane, water, and cold acetone, and used for a new batch of epoxidation.

Conclusions

A simple and green oxidation system containing H_2O_2, lactone, and lipase has been developed and successfully used for the epoxidation of many alkenes with 87–95% yields. The new oxidation system utilizes lactone as a substrate for lipase-catalyzed perhydrolysis to produce hydroxy peracid and as a solvent for the *in situ* chemical oxidation. Different from other lipase-mediated oxidation systems, no short-chain acid and alcohol, which are harmful to enzyme activity, is generated during the formation of hydroxy peracid. The mechanism for the oxidation system has been proposed and confirmed. The new system has demonstrated several advantages such as higher yield, higher efficiency, and higher enzyme stability over other lipase-mediated oxidation systems including H_2O_2/ester, H_2O_2/carbonate, and UHP/ethyl acetate. The easily available hydrophilic ε-caprolactone and hydrophobic δ-decanolactone have been successfully applied in such a system, leading to the practical generation of different medium-chain hydroxy peracids as well as the choice of using a single or two-liquid phase for desired oxidations. The green oxidation system developed here has been proven to be very useful for the epoxidation of alkenes, and it could be applicable to other types of oxidations.

Medium-chain alkyl peroxy acids such as 6-hydroxy-percaproic acid was known to be very effective for disinfection/bleaching,[2,10a] and such peroxy acid is currently prepared from ε-caprolactone by using very strong acid.[10b] Our method provides a green and practical alternative for producing this kind of hydroxy peracid, thus being useful for disinfection/bleaching.

Experimental

Materials

Novozym 435® was bought from Novozymes. It is *Candida Antarctica* lipase B immobilized on a macroporous acrylic resin. The specific activity for Novozym 435® is 10,000 PLU/g catalysts, meaning 10,000 U/g catalysts for the synthesis of propyl laurate (PL) from lauric acid and 1-propanol. ε-Caprolactone (99%), styrene (>99%), styrene oxide (98%), 4-chlorostyrene (97%), 4-chlorostyrene oxide (96%), α-methylstyrene (99%), cyclohexene (>99.5%), cyclohexene oxide (98%), α-pinene (98%), α-pinene oxide (97%) were purchased from Sigma-Aldrich. δ-Decanolactone (>99%) was obtained from Wako (Japan). Ethyl acetate (>99%) was bought from Merck. α-Methylstyrene oxide (97%) was obtained from TCI (Japan). 6-Hydroxycaproic acid (95%) was purchased from Alfa Aesar.

Analytical methods

The concentrations of olefins and corresponding epoxides were determined using a HP-5 column (Agilent, 30 m × 0.32 mm) connected to an Agilent 7890A gas chromatograph (Agilent, USA) with splitless injection and a temperature profile for cyclohexene and cyclohexene oxide: 40 °C for 1 min, then to 140 °C at 12 °C min^{-1}, and finally to 280 °C at 50 °C min^{-1}. Retention times: 3.6 min for cyclohexene, 5.6 min for cyclohexene oxide, 9.0 min for ε-caprolactone, 9.3 min for *n*-dodecane (internal standard), 9.5 min for 6-hydroxycaproic acid. For other olefins and corresponding epoxides, the temperature profile is: 100 °C for 1 min, then to 170 °C at 10 °C min^{-1}, and finally to 280 °C at 50 °C min^{-1}. Compounds were detected by a flame ionization detector with helium as the carrier gas. Retention times: 2.9 min for styrene, 4.2 min for styrene oxide; 4.3 min for 4-chlorostyrene, 6.2 min for 4-chlorostyrene oxide; 3.5 min for α-methylstyrene, 4.4 min for α-methylstyrene oxide; 3.2 min for α-pinene, 4.6 min for α-pinene oxide, 5.0 min for ε-caprolactone, 5.4 min for *n*-dodecane (internal standard), 5.9 min for 6-hydroxycaproic acid.

General procedure for the epoxidation of different alkenes with hydrogen peroxide, ε-caprolactone, and lipase

A mixture of alkene (1 mmol), 50% hydrogen peroxide (2.5 mmol), K_2HPO_4 (0.5 mmol), Novozym 435® (20–40 mg) and ε-caprolactone (1.5 mL) was shaken in a test tube closed with a cap at 300 rpm and 30 °C for different reaction time. The reaction was followed by taking samples at different time-points. 10 μl of solution was diluted 100 times by mixing with 990 μL ethyl acetate containing 2 mM *n*-dodecane as internal standard. The sample was analyzed by GC.

Lipase-catalyzed hydrolysis or perhydrolysis of ε-caprolactone

2.5 mmol of 50% hydrogen peroxide (containing 4.7 mmol H_2O) or 4.7 mmol H_2O was added to ε-caprolactone (1.5 ml). The reaction was started after adding 20 mg of Novozym 435® to the mixture. The mixture was shaken in a test tube closed with a cap in a shaker at 300 rpm and 30 °C for 1 h. The concentration of produced 6-hydroxycaproic acid was determined by GC. 179 mM and 335 mM of 6-hydrocaproic acid were obtained after 1 h by adding H_2O_2 and H_2O, respectively.

Lipase-mediated epoxidation of cyclohexene with hydrogen peroxide in acetonitrile using 6-hydroxy caproic acid or ε-caprolactone as perhydrolysis substrate

To a solution of the cyclohexene (1 mmol), 50% hydrogen peroxide (2.5 mmol), 6-hydroxy caproic acid (0.2 mmol) or ε-caprolactone (0.2 mmol) in acetonitrile (1.5 mL) was added Novozym 435® (20 mg). The mixture was shaken in a test tube closed with a cap in a shaker at 300 rpm and 30 °C for 1 h to determine the initial reaction rate. The concentration of cyclohexene and cyclohexene oxide was determined by GC. The epoxidation activity was 91.7 μmol min^{-1} g^{-1} Novozym 435® by using 6-hydroxy caproic acid and 1.5 μmol min^{-1} g^{-1} Novozym 435® by using ε-caprolactone.

Epoxidation of cyclohexene with urea-hydrogen peroxide (UHP), ε-caprolactone, and lipase

To a solution of the cyclohexene (1 mmol) in lactones (1.5 mL) were added urea hydrogen peroxide (2.5 mmol) and Novozym 435® (20 mg). The mixture was shaken in a test tube closed with a cap in a shaker at 300 rpm and 30 °C for different reaction time. The reaction was followed by analyzing samples taken at different time points by GC. Cyclohexene oxide was formed in 22.9% (154 mM) at 1 h and 62.0% (415 mM) at 4 h.

Epoxidation of styrene with hydrogen peroxide and lipase in δ-decanolactone or ethyl acetate: Enzyme recycling

A mixture of styrene (1 mmol), 50% hydrogen peroxide (2.5 mmol), Novozym 435® (50 mg) in ethyl acetate (1.5 mL) or δ-decanolactone (1.5 mL) was shaken in a test tube closed with a cap in a shaker at 300 rpm and 30 °C for 48 h. For the latter case, 1 mmol K_2HPO_4 was added. After 48 h, the solution was filtered and the solid was firstly washed with hexane (or ethyl acetate) to remove product, then DI water to remove the salt or acid, and finally cold acetone to remove water absorbed on enzyme. The dried enzyme was added to a mixture of fresh substrate and solvent (δ-decanolactone or ethyl acetate) to start a new batch. The concentration of styrene and styrene oxide was determined by GC.

Acknowledgements

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