Catalysis Communications 11 (2010) 727–731

Contents lists available at ScienceDirect

Catalysis Communications

journal homepage: www.elsevier.com/locate/catcom

Synthesis of chiral epichlorohydrin by chloroperoxidase-catalyzed epoxidation of 3-chloropropene in the presence of an ionic liquid as co-solvent

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ARTICLE INFO

Article history:
Received 8 October 2009
Received in revised form 4 February 2010
Accepted 4 February 2010
Available online 11 February 2010

Keywords:
Chloroperoxidase
Asymmetric epoxidation
Chiral epichlorohydrin
Ionic liquids

ABSTRACT

Asymmetric epoxidation of 3-chloropropene can be catalyzed by chloroperoxidase (CPO) from *Caldariomyces fumago* to prepare (*R*)-epichlorohydrin (ECH) in homogenous phosphate buffer/ionic liquid mixtures using *t*-butyl hydroperoxide (TBHP) as O_2 donor.

Reaction conditions were optimized by the investigation of the choice of oxidants, the presence of ionic liquids (ILs), pH effect and CPO consumption. The best ECH yield reached 88.8% within a duration of 60 min with high enantiomeric excesses (e.e. 97.1%) at pH 5.5 and room temperature, using 1-ethyl-3-methylimidazolium [EMIM][Br] as co-solvent. The ILs with shorter carbon chain was more efficient on chiral ECH preparation.

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1. Introduction

The synthesis of small chiral fragments attracted considerable interest due to their potential application. Chiral epichlorohydrin (ECH) was used widely in organic synthesis and medicinal chemistry. For example, it is very easy to use chiral epichlorohydrin to generate optically active compounds, such as antihypertensive and antianginal agents.

The original chemical preparation of chiral ECH was reported in 1978 [1], but the synthetic process was considered cumbersome and impractical. Unlike the traditional chemistry method, biocatalysis presents a straightforward and environment-friendly approach for the generation of this chiral monomer. However, the efforts on the asymmetric synthesis of ECH catalyzed by whole cells, such as *Rhodotorula glutinis, Aspergillus niger* and *Arthrobacter erithii H10A*, were less successful due to low product yields were observed [2–4].

Chloroperoxidase (CPO) is the most synthetically useful peroxidase due to its flexibility to a wide range of organic substrates as well as its ability of catalyzing a variety of different reactions. More importantly, chloroperoxidase is able to catalyze a broad spectrum of enantioselective reactions, such as epoxidations of olefins, hydroxylations of benzylic or allylic carbons, oxidations of alcohols, sulfides and indole [5,6].

However, the use of CPO was limited due to its poor activity and stability in solvents with low water content. Recent years, ionic liquids have been investigated as alternative solvents for biocatalysis in lots of studies. Remarkable results were obtained for lipase and other peroxidases catalysis in pure ionic liquid or homogeneous ionic liquid/water mixtures [7–9].

In this work, we describe the strategies of chiral ECH preparation by CPO-catalyzed epoxidation of 3-chloropropene in the presence of an ionic liquid as co-solvent. So far as we know, this is the first report of the chiral ECH preparation by biocatalysis in ionic liquid–water mixtures.

2. Materials and methods

2.1. Materials

Chloroperoxidase was isolated from the growth medium of *Caldariomyces fumago* according to the method established by Morris and co-workers [10] with minor modifications, using acetone rather than ethanol in the solvent fractionation step. The enzyme had a specific activity of 4800 U/mL based on the standard monochlorodimedone (MCD) assay ($Rz = 1.05$).

tert-Butyl hydrogen peroxide (TBHP), potassium hydrogen phosphate, potassium dihydrogen phosphate, hydrogen peroxide (30% in aqueous solution), dimethylsulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), methanol (CH_3OH), acetonitrile (CH_3CN) and acetone (CH_3OCH_3) were obtained from Xi'an Chemical Co. Ltd. 3-Chloropropene, 1-ethyl-3-methylimidazolium [EMIM][Br],

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doi:10.1016/j.catcom.2010.02.003

1-propyl-3-methylimidazolium [PMIM][Br], 1-butyl-3-methylimidazolium [BMIM][Br], and 1-amyl-3-methylimidazolium [AMIM][Br] were purchased from Aldrich. All chemicals are of analytical grade unless otherwise indicated. The standard epichlorohydrin was obtained from Aldrich at a stated purity of 99.0%.

2.2. Procedure for enzymatic epoxidation of 3-chloropropene

3-Chloropropene (0.3 mmol) and CPO (0.04 μmol) were magnetically stirred in 3.0 mL 0.1 M aqueous phosphate buffer at room temperature, pH 5.5. Then, TBHP (0.6 mmol) was added directly. The reaction was quenched after 60 min with a saturated sodium sulfite solution and extracted three times by anhydrous ether. Combined organic extracts can be purified by evaporation, and was collected at 88 °C and dried with magnesium sulfate.

The epoxidation using ionic liquids as co-solvent was performed under the same conditions as above.

2.3. GC analysis and determination of product yield and enantiomeric excess

Chiral gas chromatography analyses were performed on a Agilent 6890N gas chromatograph equipped with a β-DEX 120 (30 m × 0.25 mm × 0.25 μm) chiral column.

GC standard (decane) was added prior to injection. Both chemical yields and enantiomeric excesses of ECH were determined in a single chromatogram based on their consistent elution order during GC analysis compared with the standard ECH. In all cases the predominant enantiomer produced was *R*-configuration.

3. Results and discussion

3.1. Optimization of conditions for CPO-catalyzed epoxidation of 3-chloropropene

The synthetic strategy (described as Scheme 1) is straightforward and applicable to large scale preparation of chiral ECH. An increasing–decreasing pattern versus reaction time was found for product accumulation (expressed as ECH yield) in aqueous phosphate buffer at room temperature. The yield reached maximum within 60 min before it started dropping, probably due to enzyme inactivation as well as spontaneous hydrolytic epoxide ring opening and aggregation of product. The reaction was rather enantioselective (e.e. > 93.9%), indicating that 3-chloropropene was a good substrate to CPO. In order to improve the chemical and optical yield, we investigated the influence of several reaction conditions, including the choice of oxidants, the presence of ionic liquids, pH effect and CPO consumption.

3.2. Effect of oxidants on CPO-catalyzed epoxidation of 3-chloropropene

While many CPO-mediated reactions involved H_2O_2 as the terminal oxidant, this work utilized TBHP instead.

CPO can be inactivated by excess H_2O_2 in the reaction mixture. This inactivation, which generally occured with heme proteins such as cytochrome P450, horseradish peroxidase and CPO, probably involved internal oxidation of the porphyrin moiety [11,12]. On the other hand, CPO has a catalase activity, which would cause spontaneous consumption of H_2O_2. Therefore, it was important to keep the H_2O_2 concentration as low as possible in reaction solution to suppress catalase activity and inactivation of CPO. A H_2O_2-controlled reaction model was often employed to improve the enzyme performance, where a prolonged reaction time (even up to 20 h) was required [13].

In this work, TBHP was chosen as the O_2 donor instead of H_2O_2. TBHP could be introduced into the reaction system directly. Accordingly, the reaction time was dramatically decreased (from over 4 h to about 60 min). Moreover, CPO was able to generate O_2 from H_2O_2 in a catalase-type side reaction, causing foaming and potentially sweeping away more volatile substrates [14]. However, the reaction, using TBHP as oxidant instead, can be performed in a sealed vessel without pressure buildup. In fact, we found that a change of oxygen source did not affect the high enantioselectivity, and moreover, a higher ECH yield was achieved even in the presence of excess oxidant.

Scheme 1. Synthesis of chiral epichlorohydrin by CPO-catalyzed epoxidation of 3-chloropropene

3.3. Effect of organic solvent

The increasing interest in the use of enzymes in synthesis identified advantages of enzymatic catalysis in organic media from those displayed in aqueous media. These advantages included enhanced solubility of hydrophobic substrates, improved substrate specificity and product enantioselectivity [15]. In this work, the effect of some widely used organic solvents on epoxidation of 3-chloropropene using CPO was investigated, such as DMSO, DMF, CH_3OH, CH_3CN and CH_3OCH_3. However, it was found that these organic solvents were not suitable because CPO epoxidation activity was inhibited in their presence (Fig. 1). This was consistent with the conclusion of Ref. [16], in which the authors reported that the chlorination rates of monochlorodimedon (MCD) using CPO in the presence of 20% DMSO, DMF, methanol or acetonitrile were only 58% of the rate in pure buffer (pH 2.8) at the same reactant concentrations. The presence of such organic solvents was found to inhibit CPO catalysis by altering the protein conformation and the local environment around the active site [16].

3.4. Effect of ionic liquids

Recent years, ionic liquids (ILs) have gained increased attention as new solvents for non-conventional biocatalysis. Remarkable results have been obtained for CPO catalysis with respect to the yield,

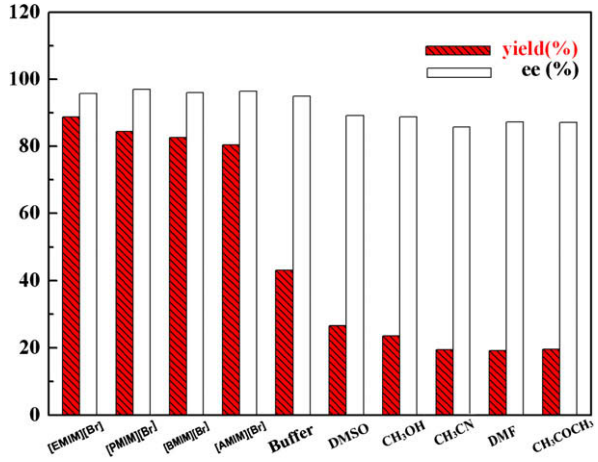

Fig. 1. Chiral ECH preparation in the presence of different organic solvent and ILs as co-solvent or in pure buffer. Reaction conditions: 0.04 μmol CPO, 0.3 mmol 3-chloropropene, 0.6 mmol TBHP, 1.6% (v/v) of ILs or organic solvent, pH 5.5.

enantioselectivity or enzyme stability [17,18]. In this work, [EMIM][Br], [PMIM][Br], [BMIM][Br], and [AMIM][Br] were considered due to that the substrate was entirely soluble in them. In addition, these ILs were miscible with water, but not with the common organic solvents, thereby permitting the recovery of products by selective extraction procedure.

Initially, the reaction was carried out with pure ionic liquid as solvent. However, a longer period was required for the product to appear. Meanwhile, an enzyme inactivation was observed. So the reaction was performed in a homogeneous reaction media formed by phosphate buffer containing 1.6% (v/v) of hydrophilic ILs as co-solvent.

As expected, all the investigated ionic liquids have positive effect. The ECH yields were enhanced to 80.4–88.8%, accompanying an improved enantiomeric excess (95.6–97.1%), compared to optimum 41.6% yield and 93.9% e.e. in pure phosphate buffer (Figs. 1 and 2). The ILs with shorter carbon chain was more efficient on chiral ECH preparation.

Successively, the ionic liquids content was extended gradually to 45% (V_{ILs}/V_{buffer}). Higher concentrations of ILs were not taken into account because an undesirable pH variation was observed,

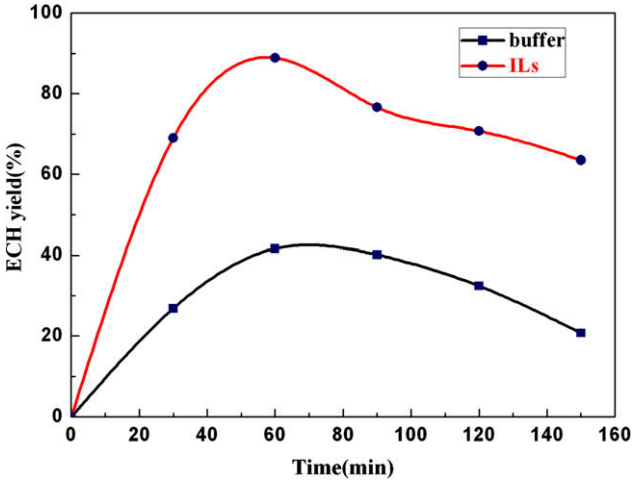

Fig. 2. Comparison of chemical yield and enantiomeric excesses of ECH in pure buffer or in the presence of 1.6% (v/v) of [EMIM][Br] as co-solvent. Reaction conditions: 0.04 μmol CPO, 0.3 mmol 3-chloropropene, 0.6 mmol TBHP, pH 5.5.

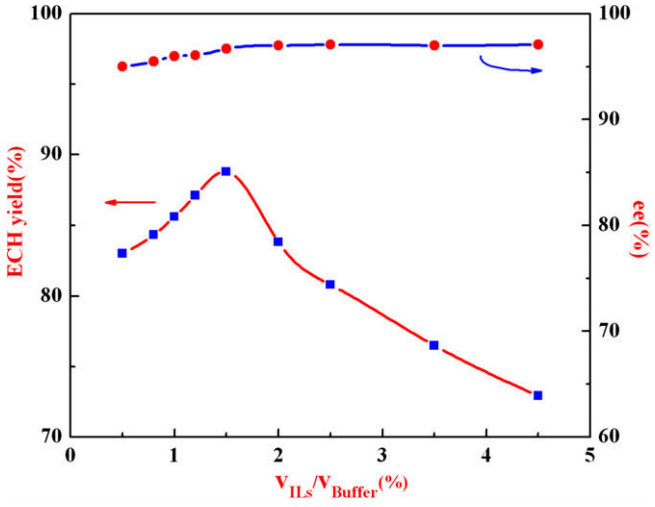

Fig. 3. Effect of [EMIM][Br] concentration on the epoxidation of 3-chloropropene catalyzed by CPO. Reaction conditions: 0.04 μmol CPO, 0.3 mmol 3-chloropropene, 0.6 mmol TBHP, pH 5.5.

Table 1
ECH chemical yields and enantiomeric excesses of the CPO-catalyzed epoxidation of 3-chloropropene with ILs as co-solvent at optimum reaction condition.

ILs	V_{ILs}/V_{buffer} (%)	ECH yield (%)	e.e. (%)
[EMIM][Br]	1.6	88.8	97.1
[PMIM][Br]	1.6	84.4	96.3
[BMIM][Br]	1.6	82.5	96.0
[AMIM][Br]	1.6	80.4	95.6

which was reported to be detrimental to the activity of CPO [19]. A "bell-shaped" dependence of ECH yields on ILs concentration was observed (Fig. 3), where [EMIM][Br] was taken as an example. Table 1 reported the optimum ILs concentrations and the corresponding ECH yields and enantiomeric excesses values.

Obviously, the detrimental effect on epoxidation of 3-chloropropene in organic media using CPO was favorably absent in the ionic liquids considered. The product yield was higher than that in pure buffer in a wide concentration range of ILs. CPO-tolerated ILs concentration was 38.7% (V_{ILs}/V_{buffer}). UV–visible spectrum of CPO in the presence of ILs showed that the absorption of CPO Soret band at λ_{max} = 398 nm was increased. No blue-shift of absorption peak was observed (Fig. 4). This phenomenon indicated that the heme was more exposure, so it was easier for CPO to bind with substrates. But a decrease of λ_{max} appeared gradually, accompanying an increase of λ = 280 nm when V_{ILS}/V_{buffer} was higher than 10%. Circular dichroism (CD) spectroscopy was employed to investigate the structure and conformation of CPO in this process (Fig. 5). In "far-UV" spectral region (190–250 nm) of CPO, there was a large negative absorption peak around 206 nm, which indicated CPO was a typical alpha-helix protein. This negative absorption became stronger when the concentration of ILs increased. This increase was due to the strengthening of alpha-helix structure of CPO. However, a loose of this alpha-helix structure was observed at higher ILs content, which was not favorable for CPO catalytic performance. In "near-UV" spectral region (250–350 nm), the increase of CD signal around 276 nm in the presence of ILs showed the corresponding change of tertiary structure, which was favorable for stability and activity of enzyme. No obvious change of CD signal at 350–700 nm was observed, meaning that the microenvironment around heme was not changed in the presence of ILs.

3.5. Effect of pH

The epoxidation of 3-chloropropene was highly pH-dependent. But it only had influence on the yield. No great changes on e.e. val-

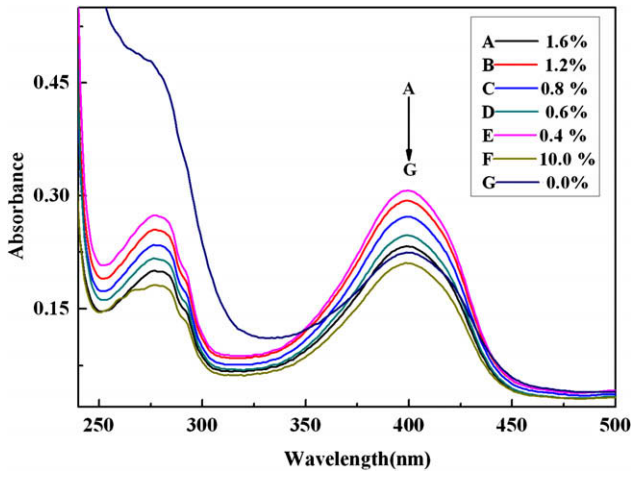

Fig. 4. UV–vis spectrum of CPO in the presence of different ILs from 1.6% to 10% V_{ILs}/V_{buffer}.

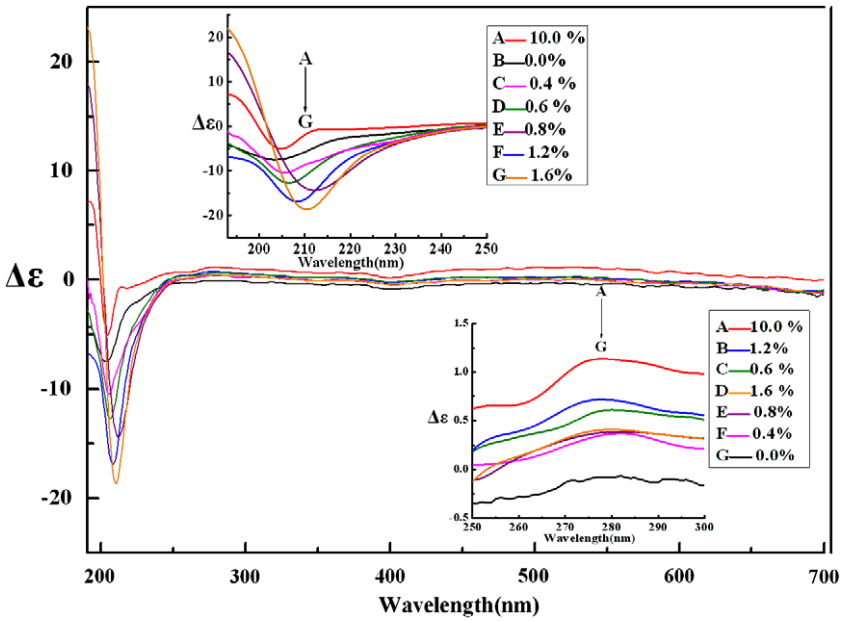

Fig. 5. CD spectrum of CPO in the presence of different ILs from 1.6% to 10% V_{ILs}/V_{buffer}.

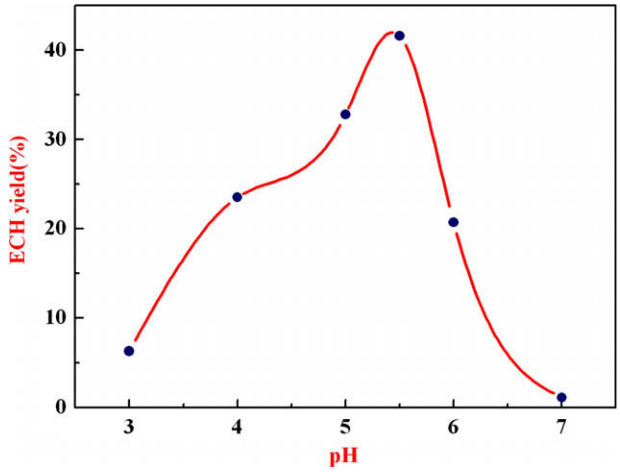

Fig. 6. Effect of pH on CPO-catalyzed epoxidation of 3-chloropropene. Reaction conditions: 0.3 mmol 3-chloropropene, 0.6 mmol TBHP, 0.04 μmol CPO.

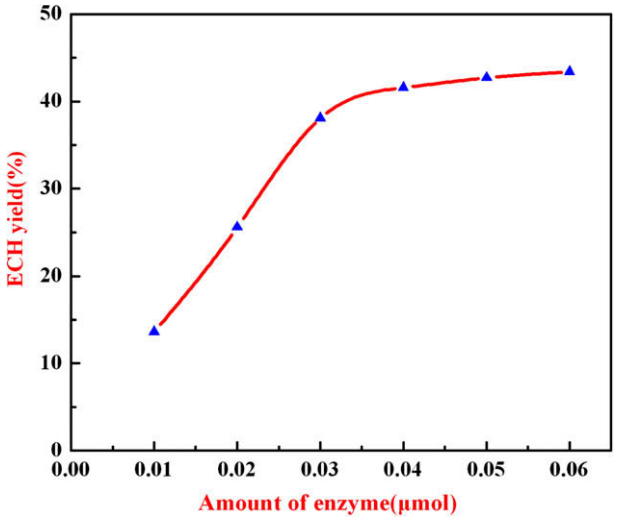

Fig. 7. Effect of enzyme consumption on CPO-catalyzed epoxidation of 3-chloropropene. Reaction conditions: 0.3 mmol 3-chloropropene, 0.6 mmol TBHP, pH 5.5.

ues were observed under different pH. The highest ECH yield was obtained at pH 5.5, as shown in Fig. 6. We did not test when pH > 7 because under alkaline conditions, CPO was extremely unstable. It would lose its activity completely in a short time. On the other hand, some side reactions will probably arise under alkaline conditions. At the range of pH 3.0–7.0, the ECH yield significantly increased with the increase of pH from 3 to 5.5, and then, dropped due to the CPO inactivation. Strong acidic condition was also not chosen because of the poor stability of enzyme.

3.6. Effect of enzyme consumption

Enzyme amount ranging from 0.01 to 0.06 μmol was employed to determine the effect of protein dosage on the ECH yield (Fig. 7). An enzyme concentration-dependent model of product yield was found. The yield firstly increased linearly with the increase of CPO amount, thereafter the increase slowed down and reached a plateau with high enantioselectivity maintained. Here, TBHP was used at least 2-fold molar excess over substrate concentration, but no inactivation of CPO was observed.

To ensure both the completion of reaction and fewer consumption of enzyme, a CPO concentration of 0.04 μmol was used in all experiments, indicating a very small enzyme amount was required in this reaction.

4. Conclusion

Chiral epichlorohydrin with high yields and enantioselectivity was prepared in this work. Under the optimum conditions, the ECH yield reached 88.8% with e.e. of 97.1%. This work shows that the enzymatic epoxidation of 3-chloropropene is a very powerful method for the production of optically active epichlorohydrin, especially with imidazole ionic liquids as co-solvent.

As compared to the behavior observed in conventional organic solvents, CPO in ILs presented enhanced activity, stability and selectivity. Moreover, the presence of ILs improved substrate solubility in the reaction medium. The ionic liquid having shorter carbon chain was more efficient on chiral ECH preparation.

The results demonstrate an interesting and practical applicability of CPO, since previously only lower enantioselectivity and chemical yields has been reported for asymmetric syntheses of ECH. As CPO from *C. fumago* is readily available, their use in the synthesis of other optically active epoxide monomers, which are difficult to obtain by chemical methods, might be a promising approach.

Acknowledgment

This work is supported by the National Natural Science Foundation of China (20876094).

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