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ORIGINAL PAPER

Application of various ionic liquids as cosolvents for chloroperoxidase-catalysed biotransformations

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Abstract Chloroperoxidase from *Caldariomyces fumago* catalyses oxidation of indole and thioanisole in reaction mixtures containing up to 40% (*v*/*v*) of different ionic liquids (ILs). Results indicate that ILs containing tosylate, trifluoroacetate, chloride, and methylsulfate anions are suitable cosolvents for these transformations, yielding high enantiomeric excess and good conversion rates.

Keywords *Caldariomyces fumago* · Sulfoxides · Chirality · Oxidation · Ionic liquids

Introduction

Ionic liquids (ILs) have gained increasing interest in recent years [1–4]. Their properties, such as viscosity, polarity, and miscibility with various solvents, can be tuned by appropriate variation of the cation and anion [5–8]. This feature, together with their lack of vapour pressure and the possibility of recycling, makes ILs excellent candidates for cosolvents in biocatalytic transformation reactions [9–11]. Applications of ILs as reaction media for enzyme catalysis have so far focussed on lipases, proteases, and glycosidases. Very few examples of oxidase or peroxidase reactions in ILs have been described in the literature [12–14].

In our studies we used chloroperoxidase (CPO) from *Caldariomyces fumago* [E.C. 1.11.1.10] as biocatalyst. This enzyme has proved to be of great value in chemical synthesis, because it tolerates a wide variety of substrates in the reactions catalysed [15, 16]. However, because of the low solubility of organic substrates in aqueous solution, many applications suffer from low yields and make the use of cosolvents necessary.

It has been demonstrated, that CPO exhibits good conversion rates in the oxidation of 1,2-dihydronaphthalene in the presence of up to 30% (*v*/*v*) 1-butyl-3-methylimidazolium methylsulfate [17]. Recently, the stability of CPO in the presence of different ILs has been confirmed using other substrate compounds [18]. These results inspired us to investigate the effect of various ILs as cosolvents in CPO-catalysed transformations, with regard to conversion rates and enantiomeric excess values. As model reactions, we chose the oxidation of indole (**1**) to oxindole (**2**), and the conversion of thioanisole (**3**) to the corresponding sulfoxide (**4**) using H_2O_2 and *tert*-butyl hydroperoxide (*tert*-BuOOH) as oxidants (Fig. 1). Both transformations are of interest in organic chemistry; in particular, the synthesis of enantioselective sulfoxides still poses a synthetic challenge [19].

Results and discussion

To examine the applicability of ILs in CPO-catalysed biotransformation we synthesized and purified a variety of these cosolvents following established methods [20–22].

Chloroperoxidase-catalysed indole oxidation in the presence of these ILs (20% *v*/*v*) using slow addition of H_2O_2 was performed as described in the Experimental section. The conversion rates of these experiments are summarized in Table 1. The results indicate that, in particular, ILs containing tosylate, trifluoroacetate, chloride, or methylsulfate anions are suitable cosolvents in this biotransformation reaction.

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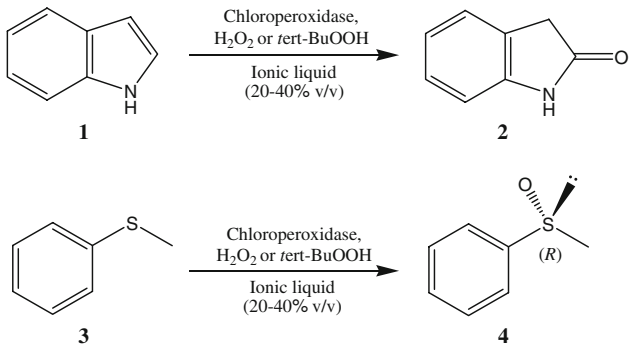

Fig. 1 Model reactions used to investigate the effect of various ionic liquids as cosolvents on CPO-catalysed transformations, with regard to conversion rates and enantiomeric excess values

Table 1 Conversion rates for indole oxidation using H_2O_2 as oxidant

Entry	Ionic liquid	Conversion (%)
1	[BMIM][ts]	100
2	[BMPY][ta]	98
3	[BMIM][ta]	97
4	[BMIM][Cl]	94
5	[BMPY][tf]	90
6	[MMIM][ms]	83
7	[BMIM][BF_4]	71
8	[BMIM][NO_3]	48
9	[BMIM][tf]	44
10[a]	[BMPY][bta]	16
11[a]	[BMIM][PF_6]	15
12[a]	[Oct_3NMe][NO_3]	12
13[a]	[BMIM][bta]	9

Reactions were performed in acetate buffer (pH 4.5) containing 20% (*v*/*v*) ionic liquid

[BMIM], 1-butyl-3-methylimidazolium; [BMPY], 1-butyl-1-methylpyrrolidinium; [MMIM], 1,3-dimethylimidazolium; [ts], tosylate; [bta], bis(trifluoromethylsulfonyl)imide; [Oct], octyl; [ta], trifluoroacetate; [tf], trifluoromethylsulfonate; [ms], methylsulfate

[a] IL not miscible with aqueous buffer

As expected, application of water-immiscible ILs led to a significant decrease in conversion rates (entries 10–13 in Table 1). The latter results led us to examine the tolerance of the ILs by CPO in respect of increasing amounts of the ILs in the reaction media. The correlation of conversion rates with IL content of the reaction mixture is summarized in Fig. 2.

The data suggest that the cosolvents applied in this reaction system can be divided into two different groups:

1 the first showed a significant decrease in conversion between 30 and 40% (*v*/*v*) cosolvent, whereas
2 the second group of ILs exhibited a rather linear decay in the conversion rates.

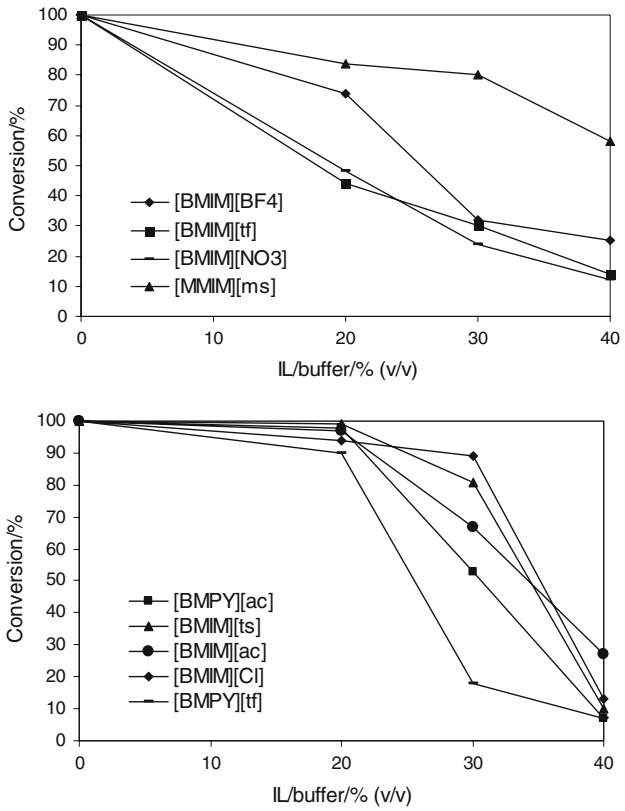

Fig. 2 Conversion rate as a function of amount of ionic liquid in the reaction mixture for CPO-catalysed oxidation of indole

In general, more than 40% IL in the reaction medium always led to poor conversions with the single exception of [MMIM][ms], which still gave 58% yield. When pure ILs were used as cosolvents for CPO-catalysed indole oxidation no reaction products were obtained.

To gain further insight into the applicability of ILs for CPO-catalysed oxidation reactions, we investigated the transformation of thioanisole (**3**) to its corresponding sulfoxide **4** as a model reaction. To obtain a general picture, we also included the most common organic cosolvents for CPO-catalysed biotransformation, *tert*-butanol and acetone, in our investigations. The results obtained from the oxidations in various ILs are summarized in Figs. 3 and 4 for use of H_2O_2 and *tert*-BuOOH, respectively, as oxidizing reagents.

As can be concluded from Fig. 3, conversion rates for this biotransformation significantly increased when ILs containing trifluoroacetate, chloride, methylsulfate, and tosylate anions were added to the pure buffer solutions. Additionally, the higher conversion rates could be reached at a lower IL content (20%) than was the case for the usually applied organic cosolvents acetone and *tert*-butanol (40%).

Changing the oxidant from H_2O_2 to *tert*-BuOOH yielded slightly different results, summarized in Fig. 4. In the case

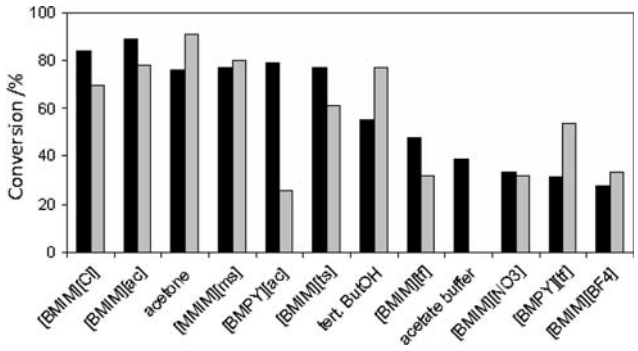

Fig. 3 Conversion rate for thioanisole oxidation with H_2O_2. Reaction mixtures contain 20% (*v*/*v*) (*black*) or 40% (*v*/*v*) cosolvent (*grey*)

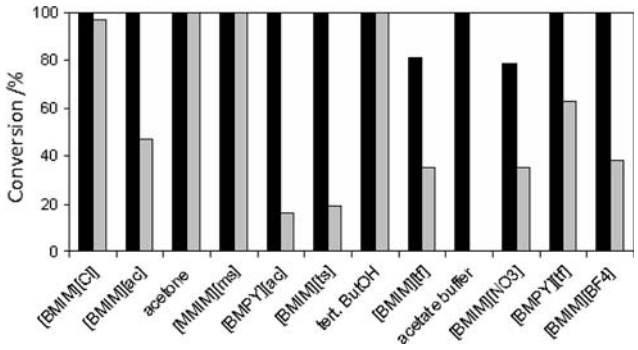

Fig. 4 Conversion rate for thioanisole oxidation with *tert*-BuOOH. Reaction mixtures contain 20% (*v*/*v*) (*black*) or 40% (*v*/*v*) of cosolvent (*grey*)

of a 20% (*v*/*v*) IL content in the solvent mixture the conversion rate was increased when changing from H_2O_2 to *tert*-BuOOH. However, the overall reaction time was also significantly raised. This fact may be responsible for the significant drop in the conversion rate observed when increasing the IL content to 40%. Obviously, longer reaction times led to lower conversion rates because of reduced enzyme stability in solvent mixtures containing larger amounts of IL. Notable exceptions to this behaviour are represented by [BMIM][Cl] and [MMIM][ms], for which, even in the presence of 40% (*v*/*v*) IL, comparable rates could be observed. All efforts to perform CPO-catalysed oxidation in pure ILs failed. The enantiomeric excess values obtained from the enzyme reaction by applying both oxidants in different solvent mixtures are summarized in Table 2.

Usually the *ee* values do not differ when the IL content of the solvent mixture is increased. One interesting exception is found when [BMIM][Cl] is applied as cosolvent. In this case 40% (*v*/*v*) IL led to significantly higher *ee* values than was observed for 20% (*v*/*v*) content, irrespective of the oxidant used. At present, we do not have a good explanation for this behaviour, which needs further investigation of solvent structure and function of the ILs and the mixtures thereof.

Table 2 Enantiomeric excess values for thioanisole oxidation in acetate buffer, containing 20 or 40% cosolvent

Cosolvent	*ee* (%)	
	20%/40% (*v*/*v*)	
	H_2O_2 oxidation	*tert*-BuOOH oxidation
Acetate buffer	98	99
[MMIM][ms]	99/99	99/95
[BMIM][ac]	97/98	97/92
[BMIM][Cl]	48/90	48/81
[BMPY][tf]	83/99	98/95
[BMIM][BF_4]	65/92	98/95
[BMIM][NO_3]	65/65	91/83
[BMIM][tf]	68/60	89/83
[BMIM][ts]	96/94	95/57
[BMPY][ac]	95/70	92/42
Acetone	99/99	99/98
tert-Butanol	99/99	99/98

The results of our model reactions demonstrate that several ILs are suitable cosolvents in biotransformations catalysed by CPO from *C. fumago*. Reaction mixtures, especially those containing chloride, trifluoroacetate, methylsulfate, and tosylate ILs show high conversion rates in thioanisole and indole oxidation. The applicability of aqueous IL reaction mixtures for CPO-catalysed bioconversions was supported by the following observations:

1 the enzyme had high activity in mixtures containing 20 and 40% of the ILs mentioned above;
2 conversion rates of thioanisole oxidation with H_2O_2 could be increased from 40% in pure acetate buffer to over 80% by addition of ILs, proving the feasibility of enhancing CPO-catalysed reaction yields by increasing the solubility of the hydrophobic reaction substrates; and, finally
3 *ee* values of thioanisole oxidation products provide evidence that CPO retains high enantioselectivity in the presence of most of the tested ILs in the reaction mixture.

Further investigation of synthetic applications of other heme peroxidases in ILs are currently in progress in our laboratories.

Experimental

Chloroperoxidase from *C. fumago* [E.C. 1.11.1.10] was purchased from Fluka (CAS: 9055-20-3) as a brown suspension in 0.1 M sodium phosphate (pH = 4). ILs were synthesized using literature protocols [20–22]. The resulting products were identified and checked for organic

impurities using ^{1}H and ^{13}C NMR spectroscopy. Inorganic salt content was qualitatively determined by addition of $AgNO_3$ and precipitation of Ag halides. Purification of the ILs was accomplished by using extraction with water (water-immiscible ILs), crystallization of inorganic impurities from dichloromethane–IL mixtures, with subsequent filtration, or filtration of IL solutions in dichloromethane over silica-gel, if necessary. CPO reactions were run in a Carousel 12 Reaction Station from Radleys and oxidant addition was controlled by an Orion M362 Sage pump. HPLC analysis was performed using a Chiralcel OD-H column (0.46 cm × 25 cm).

Indole oxidation

In a typical procedure for indole oxidation, 10 mg substrate **1** was dissolved in 1.5 cm^3 of the corresponding solvent mixture containing acetate buffer (pH = 4.5; 50 mM) and the corresponding IL in a defined *v*/*v* ratio. After addition of CPO (80 U; as a suspension in a 0.1 M phosphate buffer with 10 U/mm^3), aqueous H_2O_2 (1.1 equiv. in 150 mm^3 water) was added over a period of 94 min (1.6 mm^3/min) using a syringe pump. Stirring was continued for a total reaction time of 4 h, followed by extraction of the reaction mixture with two 20 cm^3 portions of ether. The combined organic layers were washed with water (3 cm^3) and evaporated to dryness. The crude solid obtained was redissolved in $CDCl_3$ to determine the conversion rate by NMR integration (using the proton signal at 6.71 ppm for indole **1** and the singlet at 3.47 ppm for oxindole **2**).

Thioanisole oxidation

In a typical procedure for thioanisole oxidation, 15 mg **3** was dissolved in the appropriate reaction mixture containing acetate buffer (pH = 4.5; 50 mM) and the IL. After addition of CPO (125 U, as a suspension) the oxidant was added (1.1 equiv. dissolved in 150 mm^3 water) over a period of 94 min using a syringe pump. After a total reaction time of 4 h, 2 cm^3 acetophenone standard solution (498.8 mg in 50 cm^3 diethyl ether) was added and the reaction mixture was extracted with diethyl ether (2 × 20 cm^3). When *tert*-BuOOH was used as an oxidant, addition was accomplished in the same way as with H_2O_2, but the total reaction time was extended to 24 h. The organic phases were combined and washed with 3 cm^3 water. The mixture was diluted with diethyl ether to a total volume of 50 cm^3. An aliquot of this solution (20 mm^3) was injected to a HPLC column and eluted with *n*-hexane–isopropanol 8:2 using a flow rate of 0.8 cm^3/min

The eluent was monitored at 256 nm at a flow rate of 0.8 cm^3/min. Retention times were: thioanisole (**3**) 5.5 min; acetophenone 5.7 min; and methylphenylsulfoxide (*R*)-**4** 8.8 min and (*S*)-**4** 10.1 min. The enantiomeric excess was assigned as reported elsewhere [23]. Conversion rates were determined via peak-area integration. The values were corrected using a factor for recovery of the internal standard and substance.

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