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Ionic liquids as a new reaction medium for oxidase–peroxidase-catalyzed sulfoxidation

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Abstract—The possibility of the application of oxidase and peroxidase as catalysts in ionic liquids is demonstrated in the chemo- and stereoselective oxidation of sulfides. The high operational stability of these enzymes in ionic liquids is reported. The substrate of glucose oxidase (glucose) and the substrate of peroxidase (sulfide) are perfectly soluble.

1. Introduction

The use of non-aqueous solvents as a medium for enzymatic reactions has been a major breakthrough for bioconversions. However, the insolubility of polar substrates in the apolar organic solvents, which are used normally, is one disadvantage of non-aqueous techniques. Ambient-temperature ionic liquids (ILs) are very promising reaction media for chemical and biochemical transformations. They have been known for a long time, but their applications as a solvent for organic synthesis were reported in the literature only within the past few years.[1–4] ILs are good solvents for a wide range of both inorganic and organic compounds. Some ILs are water-immiscible and have polarities comparable to short-chain primary alcohols.[5–8] ILs have been already used as reaction media for lipase-catalyzed esterifications, ammonolysis of esters and perhydrolysis.[9–11] Oxidoreductases have also been the subject of some studies.[12,13] Results on biocatalysis in ILs have been reported in the recent reviews.[1,14] A different or increased selectivity was often observed by comparison to conventional organic solvents. On the other hand, ILs can enhance the catalytic activity of transition metal complexes, due to their combination of high polarity and low coordination power for the catalyst.[15,16] This non-coordinating nature might be interesting for oxidations catalyzed by heme-containing enzymes by stabilizing the highly charged iron-peroxo or -oxo intermediate. The activities of heme-derived catalysts (hemine, MP-11) are markedly higher in ILs than in methanol or DMSO.[12] Moreover, reactions in Ils have potential environmental advantages since ILs are non-volatile and can usually be recycled in good yield and reused.[3,4]

Having devised oxidation reactions catalyzed by bi-enzymatic systems in water (which failed in organic solvents) we were interested in the study of the activity of such systems in ionic liquids. Thus, we wish to report our preliminary results on the oxidation of thioanisoles by a bi-enzymatic system glucose oxidase–peroxidase in ionic liquids.

Peroxidases are very attractive biocatalysts for selective oxidative transformations, but their low operational stability limits their application.

Peroxidases are inactivated by an excess of hydrogen peroxide, their primary substrate. Only a slow flux of hydrogen peroxide can insure the stereoselectivity of reactions catalyzed by these enzymes. In our previous work, hydrogen peroxide was progressively generated in situ at the expense of glucose and oxygen (air) by glucose oxidase and used by a peroxidase to oxidise sulfides to chiral sulfoxides.[17] Co-immobilization of peroxidase with an oxidase[18] and employment of peroxidase suspended in methanol[19] and chloroform[20] were also experimented by other authors to allow recovering of the expensive POD.

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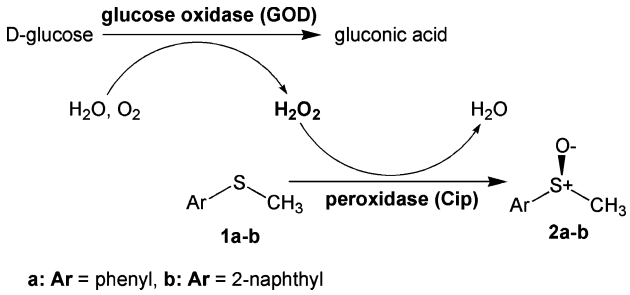
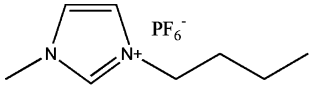


Scheme 1.

Since oxygen and organic substrates are more soluble in ILs than in water,[2,21] glucose oxidase–peroxidase-catalyzed enantioselective sulfoxidation of thioanisoles in IL as the reaction medium appeared to be a promising alternative to aqueous solution (Scheme 1).

We used for our investigations two commercially available and inexpensive enzymes: peroxidase from *Coprinus cinereus* (Cip)[22] and glucose oxidase (GOD) from *Aspergilius niger*.

We have chosen as a solvent 1-butyl-3-methyl imidazolium hexafluorophosphate ([BMIM]PF_6) a hydrophobic ionic liquid, immiscible with water and diethyl ether, but easily miscible with organic solvents such as AcOEt or CH_2Cl_2.



These properties facilitated us an easy recycling of the ionic liquids with or without enzymes as well as the extraction of sulfides and sulfoxides. Synthesis and purification of the IL was proceeded according to a modified Kaslauskas' method.[23] The use of expensive silver salts and column chromatography were avoided.

Our attempts on the direct oxidation of thioanisole by a slow addition of hydrogen peroxide to a suspension of Cip in IL failed. H_2O_2 was rapidly decomposed by the enzyme acting as a catalase under these conditions and no sulfoxide was formed.

Efficiency of enzyme-catalyzed reactions in non-aqueous media (organic solvents or ionic liquids), as it has been demonstrated in literature,[24,25] is under the control of water activity rather than water concentration. Thus, we found that the rate of production of hydrogen peroxide by GOD in IL was influenced by the amount of water added (Fig. 1). It was estimated by measuring spectrophotometrically the disappearance of glucose.[26]

The influence of the concentration of water in [BMIM]PF_6 on the conversion of thioanisole to sulfoxide catalyzed by the bi-enzymatic system GOD/Cip is illustrated in Figure 2.

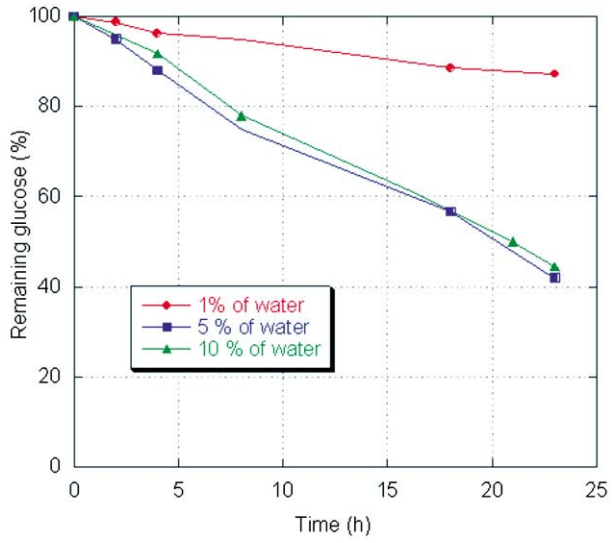


Figure 1. GOD-catalyzed oxidation of glucose in [BMIM]PF_6 as a function of concentration of water. *Conditions*: 3 mg GOD and 0.6 mmol glucose in 3 ml IL, rt.

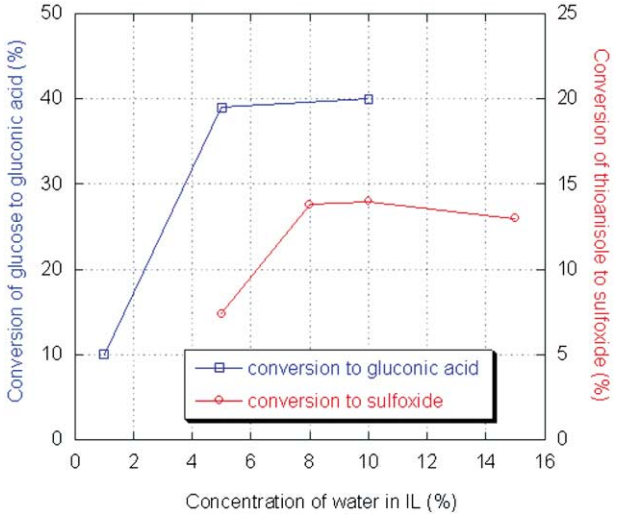


Figure 2. Conversion of thioanisole to phenyl methyl sulfoxide and of glucose to gluconic acid after 16 h of reaction catalysed by GOD/Cip at rt. *Conditions*: 0.3 mmol thioanisole in 3 ml of IL (0.1 M), 3 mg GOD, 0.6 mmol glucose, 48 mg Cip.

It appears that a 10% v/v content of water in IL is the best choice for Cip and GOD, while 5% v/v is sufficient to achieve an optimum activity of GOD. Glucose oxidase is markedly active even at 1% of water. In the optimum conditions for peroxidase, GOD activity in IL is about 20 times lower than in water.

The yield of sulfoxide relative to the hydrogen peroxide produced does not exceed 50%. Production of hydrogen peroxide by GOD increased dramatically at 40°C (Fig. 3) but resulted in low yield and low enantiomeric excess of the formed sulfoxide (Table 1, entry 4).

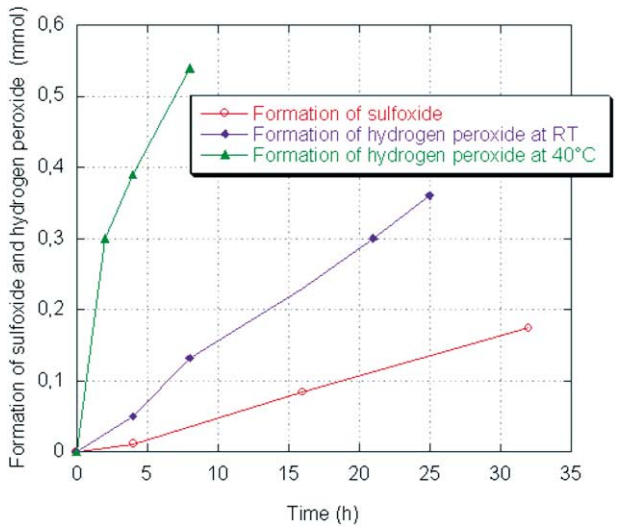


Figure 3. Rates of formation of phenyl methyl sulfoxide and hydrogen peroxide (consumption of glucose) during oxidation by GOD/Cip in [BMIM]PF_6. *Conditions*: 0.3 mmol thioanisole in 3 ml of IL (0.1 M), 0.3 ml water, 3 mg GOD, 0.6 mmol glucose, 48 mg Cip.

This probably indicates an inhibition of peroxidase by excess of hydrogen peroxide and the partial decomposition of H_2O_2 by Cip, due to its catalase activity. The low enantiomeric excess of sulfoxide obtained when an excess of GOD was used (Table 1, entry 5) shows that spontaneous oxidation of sulfide by hydrogen peroxide is in competition with that catalyzed by Cip.

Two arylmethyl sulfides: thioanisole **1a** and methyl-2-naphthyl sulfide **1b** were tested as substrates of the peroxidase. Peroxidase and glucose oxidase were added at once to the solution of thioanisole and glucose in ionic liquid–water mixture. Owing to the good solubility of sulfide and sulfoxide in IL, the reaction mixture was stirred in open vessel under air. No evaporation of substrate or product which often occurred with suspensions in water was observed. One equivalent of sodium bicarbonate, added in three portions, was required to neutralize the formed gluconic acid. Sulfide and sulfoxide were extracted by diethyl ether and enzymes in IL were recycled directly. The enantiomeric excess of the obtained sulfoxides is comparable to those in water and stay constant during the reaction. No further oxidation to sulfone was observed. The obtained chiral arylmethyl sulfoxides have the *S* absolute configuration as in water.[17]

In water, methyl-2-naphthyl sulfide **1b** (water insoluble, solid compound) was oxidized significantly slower than thioanisole.[17] On the contrary, in IL, the rate of oxidation of **1a** and **1b** were comparable (Table 1, entry 9), probably because both compounds have similar solubility in IL. Methyl-2-naphthyl sulfoxide **2b** was obtained with ee = 92%. After 48 h of peroxidase pre-incubation in [BMIM]PF_6 its activity was unchanged (Table 1, entry 7). Due to the high operational stability of enzymes in [BMIM]PF_6, after 32 h sulfoxide and its substrate could be extracted from the reaction mixture and a new portion of sulfide could be added. This second run proceeded with the same rate and with the same stereoselectivity (Table 1, entries 2 and 10), indicating that the enzymes were fully active in IL after 64 h. Thus, the reduced rate of sulfoxidation in comparison to that in water is not a consequence of the weak stability of enzymes but rather of a lower value of the kinetic constant k_{cat}. In IL 0.1 mmol of formed sulfoxide inhibits Cip and extraction of this product is indispensable to obtain complete conversion. Pure [BMIM]PF_6 can be easily recovered after enzymes, glucose and gluconic acid extraction with water.[27]

Table 1. Asymmetric oxidation of arylmethyl sulfides **1a** and **1b** catalysed by GOD/Cip in [BMIM]PF_6

Entry	Sulfide	Glucose (mmol)	GOD (mg)	Water added to IL (%)	Temp. (°C)	Time (h)	Conv. (%)	Ee (%)
1	**1a**	0.6	3	10	rt	16	13	66
						32	32	68
2[a]	**1a**	0.6	3	10	rt	16	16	nd
						32	28	67
3	**1a**	0.6	1.5	10	rt	16	7	69
4	**1a**	0.6	3	10	40	16	3	46
						32	5	nd
5	**1a**	0.6	6	10	rt	16	11	nd
						32	25	13
6	**1a**	0.3	3	10	rt	16	14	67
						32	15	nd
7[b]	**1a**	0.6	3	10	rt	16	14	66
8	**1a**	0.6	3	5	rt	16	6	63
9	**1b**	0.6	3	10	rt	16	14	92
						32	36	91
10[a]	**1b**	0.6	3	10	rt	32	34	91

Reaction conditions: sulfide **1a**, **1b**: 0.3 mmol, peroxidase: 48 mg, [BMIM]PF_6: 3 ml.

[a] Second run with enzymes–ionic liquid mixture directly recycled.

[b] Peroxidase pre-incubated in [BMIM]PF_6 for 48 h at rt.

2. Preparation of 1-butyl-3-methyl imidazolium hexafluorophosphate ([BMIM]PF_6)

1-Butyl-3-methyl imidazolium bromide ([BMIM]Br) (0.4 mol) was added to a suspension of $NaPF_6$ (0.44 mol) in acetone (200 ml). The mixture was stirred 30 h at room temperature. The precipitate of sodium bromide was removed and the filtrate was concentrated under vacuum. The residue was dissolved in ethyl acetate (200 ml) and washed a few times with sodium bicarbonate and then with water. The organic solution was dried over anhydrous sodium sulfate and ethyl acetate was removed under vacuum, to yield a pale yellow oil of 1-butyl-3-methyl imidazolium hexafluorophosphate (yield 85%).

3. Typical procedure for oxidation of sulfides

Enzymes: fungal peroxidase from *Coprinus cinereus* (EC 1.11.1.7; specific activity: 231 kU/ml) having broad pH optimum (5–9) and glucose oxidase (EC 1.1.3.4; specific activity: 1.2 U/mg) from *Aspergilius niger* with pH optimum 5.5–7. Both enzymes were supplied by Novozymes.

The sulfide (0.3 mmol) and glucose (0.6 mmol) were dissolved in a mixture of 3 ml [BMIM]PF_6 and 0.3 ml (16.6 mmol) of water containing 3 mg of glucose oxidase and 48 mg (1 mol) of peroxidase (previously dialyzed against 0.5 M NaCl and then lyophilized). The mixture was stirred mechanically at room temperature; 16 mg of sodium bicarbonate, were added every 8 hours. After 32 h the formed sulfoxide was extracted with diethyl ether. The enantiomeric excess was determinated by HPLC on a Chiracel OD-H column (flow: 0.75 ml/min, solvent: *iso*-hexane/propan-2-ol 95:5). The degree of conversion and chemical purity of sulfoxides was determinated by GC and NMR.

4. Conclusions

The devised oxidation of thioanisoles in ionic liquid by GOD/Cip leads to sulfoxides with a stereoselectivity similar to that obtained in water. All substrates of enzymes and their products: glucose, gluconic acid, sulfides and sulfoxides are perfectly soluble in IL. Thus, the reaction in IL is particularly interesting for water insoluble products (for example methyl-2-naphthyl sulfide oxidized with ee = 92%). The mixture of IL containing GOD and Cip can be quantitatively recycled and reused. Isolation of obtained sulfoxide is easier than from water, where proteins induce formation of emulsion with organic solvent. Moreover, the easy recovery of ionic liquid is environmentally interesting. These observations indicate that IL are suitable media for biocatalytic oxidations.

Acknowledgements

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27. Before washing with water [BMIM]PF_6 should be dissolved in ethyl acetate to facilitate this process.