Enzyme catalysis in ionic liquids: lipase catalysed kinetic resolution of 1-phenylethanol with improved enantioselectivity

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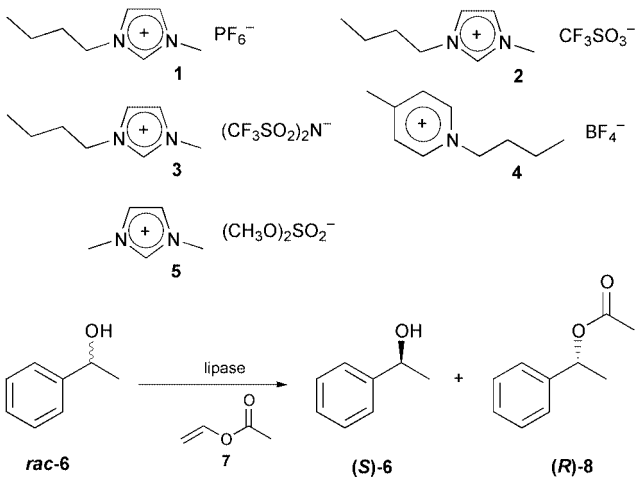
Lipases show good activity and, in some cases, improved enantioselectivity when employed in pure ionic liquids for dynamic kinetic resolution of 1-phenylethanol by transesterification.

Today, more than 100 one-step biotransformations making use of whole cells or isolated enzymes are employed on an industrial scale, including a very recent process established by BASF for the kinetic resolution of chiral amines using lipases.[1,2] On a lab scale more than 13000 enzyme-catalysed reactions have been described.[3,4] Nevertheless, there are still problems with substrate solubility, yield or (enantio-)selectivity. Some progress has been made by addition of organic solvents,[5] addition of high salt concentrations[6] or use of microemulsions[7] or supercritical fluids.[8] Recently, ionic liquids (IL) have gained increasing attention for performing all types of reactions with sometimes remarkable results.[9–11] By modification of the cation and anion their properties can be tuned in many ways. For all catalytic processes, there are basically three modes of operation: use of the IL as a co-solvent, as pure solvent or in a biphasic system. After the first trials using ethylammonium nitrate in salt water mixtures more than 15 years ago,[12] recently the first results of the use of pure ILs as a reaction medium for enzymatic reactions have been published.[13,14] A biphasic system containing an IL for *in situ* product extraction for a whole cell process has been described as well.[15]

In this paper we report our results about the application of lipases for an enantioselective reaction in pure ILs based on 1-butyl-3-methylimidazolium (BMIM) ions such as [BMIM]PF_6 **1**, [BMIM]CF_3SO_3 **2** and [BMIM]$(CF_3SO_2)_2N$ **3** or on *N*-butylpyridinium ions such as [4-MBP]BF_4 **4**. As a model system the kinetic resolution of *rac*-1-phenylethanol **6** by transesterification with vinyl acetate **7** was investigated (Scheme 1). Our results on the β-galactosidase catalysed synthesis of *N*-acetyllactosamine are reported elsewhere.[16] In that case, addition of 25% v/v of [MMIM]$MeSO_4$ **5** as a water-miscible co-solvent suppresses the secondary hydrolysis of the formed product resulting in doubling the yield to almost 60%! The reaction investigated here yields two products: the remaining alcohol **(*S*)-6** or the formed acetate **(*R*)-8**. Unless stated otherwise the enantioselectivity is always given for the (*R*)-acetate. When the enzyme shows low enantioselectivity it is not possible to reach high ee-values for the (*R*)-acetate whereas for the (*S*)-alcohol high ee is possible at the expense of the yield.



Scheme 1

A set of nine lipases and two esterases (Roche Diagnostics Chirazyme Screening Set 2) was screened for activity in ten different ionic liquids.[17] The results were compared with the reaction performed in methyl *tert*-butyl ether (MTBE) as solvent. MTBE is widely used as solvent for transesterification in industry and academia. Therefore it was used for this study despite the fact that some other solvents might be favourable as well.

The data are summarised in Table 1. Under the conditions employed the two pig liver esterases showed no activity. Best results were obtained with *Candida antarctica* lipase B (L-2) and *Pseudomonas* sp. lipase (L-6) in several ionic liquids. There is no 'best ionic liquid' in general, but [BMIM]$(CF_3SO_2)_2N$ seems to have some advantages. Surprisingly, with the lipases from *Pseudomonas* sp. and *Alcaligenes* sp. (L-10) the enantioselectivity for the formation of the acetate **(*R*)-8** is improved to a large extent compared to the reaction in MTBE. For the *Candida antarctica* lipase A (L-5) the opposite is observed. The increase of enantioselectivity is reproducible under different conditions. It is likely that the ILs will interact with charged residues found in or near the active centre of the enzyme. For two of the enzymes, L-2 and L-6 concentrations and enantioselectivity were followed as a function of time in MTBE, [BMIM]CF_3SO_3 and [BMIM]$(CF_3SO_2)_2N$. The reaction velocity is equal in both media, MTBE or IL.

One of the major advantages of ILs is that they are not volatile. Therefore it is possible to remove the products by distillation and repeat the catalytic cycle after addition of fresh substrate. This was investigated for L-2 in [BMIM]-$(CF_3SO_2)_2N$. The lipase shows good thermal stability up to 100 °C in MTBE as well as in the IL. Unfortunately, the substrate chosen and the product have boiling points around 200 °C at atmospheric pressure. Therefore, even at a pressure of 0.06 mbar a temperature of 85 °C was necessary to remove the reactants. But the enzyme suspended in the IL could be reused three times with less than 10% loss of activity per cycle. The enantioselectivity was not influenced. Certainly the recycling of the ionic liquid–enzyme system would be easier for other substrates as well as on a larger scale. These aspects as well as the improved enantioselectivity are subject to further studies. Additionally, other factors such as the water content, the viscosity or the question of which of the ions is responsible for the effects will be investigated as well.

The results presented here clearly demonstrate the potential of ionic liquids for enzymatic biotransformations. The variations possible for tailor-made solvents may have a similar impact as the pioneering work of the use of enzymes in pure organic solvents.[18]

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Table 1 Results of the screening of lipases in various ionic liquids. Conversion (%) of **rac-6** and ee's (%) of **(*R*)-8** (in brackets) are given. Conversion and ee were estimated by HPLC. Purified L-3 is also part of the Roche Chirazyme screening set

	Enzymes								
Solvent	L-2[h]	L-3[i]	L-3 purified	L-5[j]	L-6[k]	L-7[l]	L-8[m]	L-9[n]	L-10[o]
MTBE	43 (> 98)	13 (47)	< 5	11 (22)	53 (84)	45 (> 98)	10 (> 98)	29 (> 98)	> 98 (0)
[BMIM]PF_6[a]	< 5	< 5	0	10 (37)	0	< 5	< 5	< 5	44 (77)
[NMIM]PF_6[b]	10 (> 98)	7 (70)	0	41 (71)	17 (> 98)	< 5	11 (> 98)	33 (> 98)	68 (14)
[BMIM]BF_4[a]	< 5	41 (> 98)	< 5	< 5	7 (53)	0	< 5	< 5	60 (81)
[HMIM]BF_4[c]	10 (> 98)	0	0	27 (34)	0	0	0	0	26 (> 98)
[OMIM]BF_4[d]	41 (> 98)	< 5	< 5	59 (13)	< 5	< 5	< 5	< 5	50 (> 98)
[4-MBP]BF_4[e]	46 (> 98)	< 5	< 5	> 98 (3)	9 (> 98)	< 5	< 5	< 5	15 (> 98)
[BMIM]CF_3SO_3[a]	50 (> 98)	< 5	< 5	44 (45)	50 (> 98)	< 5	9 (> 98)	< 5	70 (82)
[BMIM]$(CF_3SO_2)_2N$[a]	50 (> 98)	10 (69)	< 5	> 98 (0)	47 (> 98)	8 (> 98)	12 (> 98)	40 (> 98)	89 (15)
[MMIM]$MeSO_4$[f]	Dark brown solution, analysis not possible								
[EMIM]benzoate[g]	Dark brown solution, analysis not possible								

[a] BMIM: 1-butyl-3-methylimidazolium. [b] NMIM: 1-methyl-3-nonylimidazolium. [c] HMIM: 1-hexyl-3-methylimidazolium. [d] OMIM: 1-methyl-3-octylimidazolium. [e] 4-MBP: *N*-butyl-4-methylpyridinium. [f] MMIM: 1,3-dimethylimidazolium. [g] EMIM: 1-ethyl-3-methylimidazolium. [h] L-2 *Candida antarctica* lipase B. [i] L-3 *Candida rugosa* lipase. [j] L-5 *Candida antarctica* lipase A. [k] L-6 *Pseudomonas* sp. lipase. [l] L-7 Pig pancreas lipase. [m] L-8 *Thermomyces lanuginosa* lipase. [n] L-9 *Mucor miehei* lipase. [o] L-10 *Alcaligenes sp.* lipase.

In a typical experiment 1 mg of lipase is added to 400 μl substrate solution containing 54 μl ***rac*-6** and 122 μl **7** in 4.4 ml ionic liquid or MTBE. The suspension is incubated for 3 d at 24 °C in a thermomixer. For analysis 100 μl of the reaction mixture are extracted with 1 ml *n*-hexane–propan-2-ol (97.5 : 2.5). The extract is analysed by HPLC using a chiral stationary Phase Chiracel OJ (Daicel). The eluent consists of 96.5% (v/v) *n*-hexane, 3% (v/v) propan-2-ol and 0.5% (v/v) ethanol with a flow rate of 1 ml min^{-1}; temperature 38 °C, UV-detection at 205 nm.

For the distillative workup 600 mg lipase L-2 (10 U mg^{-1}) is mixed with 4 ml [BMIM]$(CF_3SO_2)_2N$, 0.7 ml **6** and 1.2 ml **7**, mixed thoroughly and incubated at 40 °C for 40 min. Non-converted starting material and **(*R*)-8** are removed by vacuum distillation at 85 °C and 0.06 mbar. After cooling down the same amount of substrates is added again and the cycle is repeated.

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