

Biocatalytic transformations in ionic liquids

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Room temperature ionic liquids are non-volatile, thermally stable and highly polar; they are also moderately hydrophilic solvents. Here, we discuss their use as reaction media for biocatalysis. Enzymes of widely diverging types are catalytically active in ionic liquids or aqueous biphasic ionic liquid systems. Lipases, in particular, maintain their activity in anhydrous ionic liquid media; the (enantio)selectivity and operational stability are often better than in traditional media. The unconventional solvent properties of ionic liquids have been exploited in biocatalyst recycling and product recovery schemes that are not feasible with traditional solvent systems.

In recent years, room-temperature ionic liquids (compounds that consist only of ions and are liquid at room temperature) have increasingly attracted attention as the green, high-tech reaction media of the future. This is mainly because of their lack of vapour pressure, their thermal stability and their widely tunable properties with regard to polarity, hydrophobicity and solvent miscibility behaviour through appropriate modification of the cation and anion. The last characteristic has earned ionic liquids the accolade of ‘designer solvents’, although it should be stressed that the design ‘rules’ still need to be clarified. The application of ionic liquids as reaction media for organic synthesis is well documented [1–5] and their synthesis has been reviewed [1,2,5]. Some ionic liquids are available from commercial sources, such as Acros (<http://www.acros.be>), Covalent Associates (<http://www.covalentassociates.com>), Merck (<http://www.ionicliquids-merck.de>), Sachem (<http://www.sacheminc.com>), Sigma-Aldrich (<http://www.sigmaaldrich.com>), Solvent Innovation (<http://www.solvent-innovation.de>) and TCI (<http://www.tciamerica.com>).

Biocatalysis in room-temperature ionic liquids has only recently been considered and mainly concerns ionic liquids that are composed of a 1,3-dialkylimidazolium or *N*-alkylpyridinium cation and a non-coordinating anion (see Table 1). The subject attracts much interest and two brief reviews have appeared already [6,7]. Here, we discuss the issues that surround biocatalysis in ionic liquids: the effects of ionic liquids on the activity and the thermal and operational stability of biocatalysts, the effects of ionic liquids on the (enantio)selectivity of biocatalytic transformations in comparison with conventional

reaction media and the design of efficient reaction procedures that use the unconventional solvent characteristics of ionic liquids.

Solvent properties of ionic liquids


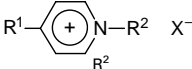
Ionic liquids generally are considered to be highly polar solvents. Solvent polarity (the propensity to solvate a charge) is a somewhat elusive issue that should not be confused with hydrophilicity, a completely separate attribute. Solvent polarity usually is determined from the absorption maximum of a solvatochromic dye (a compound with a visible absorption maximum that depends on the polarity of the solvent), such as Nile Red [8] or Reichardt’s dye [9], or by using a fluorescent probe [10]. The polarity of the ionic liquid types that we are discussing, such as [BMIm][BF₄], is in the range of 0.6–0.7 on the normalised polarity scale, which sets tetramethylsilane at 0.0 and water at 1.0 [9]. This puts ionic liquids in the polarity range of the lower alcohols [11–13] or formamide [9]. The effects of the alkyl group on the imidazole ring (C₄–C₈) and the anion (tetrafluoroborate, hexafluorophosphate, bis(trifluoromethanesulfonyl)amide) on the polarity seem to be slight and depend on the method chosen.

The miscibility of ionic liquids and water varies widely and unpredictably. [BMIm][BF₄] and [BMIm][MeSO₄] are water-miscible but [BMIm][PF₆] and [BMIm][Tf₂N], which are of similar polarity as the tetrafluoroborate, are not. Even in methanol, water does not mix at a molecular level but mainly is present as strings or clusters of molecules [14]. We assume that similar effects have a role in mixtures of ionic liquids and water. It is worth noting that water-immiscible ionic liquids are hygroscopic and dissolve up to 1% of water [15]. Some of this water is retained on drying, which could affect the physical properties [16].

The miscibility behaviour of ionic liquids and organic solvents is not well documented. A relationship with the dielectric constant has been proposed as lower alcohol and ketones, dichloromethane and THF ($\epsilon = 7.58$) mix with, for example, [BMIm][Tf₂N], whereas alkanes and ethers do not; ethyl acetate seems a borderline case [17]. On the basis of the thermodynamic activity coefficients [18] it would seem that benzene, toluene and styrene (but not the higher alkylbenzenes) dissolve in [BMPy][BF₄]. Super critical carbon dioxide (scCO₂) does not mix with ionic liquids, such as [BMIm][PF₆] and [OMIm][BF₄], but is absorbed in the ionic liquid phase in huge amounts

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Table 1. Ionic liquids discussed in this review

1-Alkyl-3-methylimidazolium cations						
Abbreviation	R					X ⁻
[MMIm][MeSO ₄]	CH ₃					CH ₃ OSO ₃ ⁻
[EMIm][BF ₄]	C ₂ H ₅					BF ₄ ⁻
[EMIm][Tf ₂ N]	C ₂ H ₅					(CF ₃ SO ₂) ₂ N ⁻
[BMIm][BF ₄]	n-C ₄ H ₉					BF ₄ ⁻
[BMIm][PF ₆]	n-C ₄ H ₉					PF ₆ ⁻
[BMIm][TfO]	n-C ₄ H ₉					CF ₃ SO ₃ ⁻
[BMIm][Tf ₂ N]	n-C ₄ H ₉					(CF ₃ SO ₂) ₂ N ⁻
[BMIm][MeSO ₄]	n-C ₄ H ₉					CH ₃ OSO ₃ ⁻
[BMIm][EtSO ₄]	n-C ₄ H ₉					C ₂ H ₅ OSO ₃ ⁻
[BMIm][NO ₃]	n-C ₄ H ₉					NO ₃ ⁻
[BMIm][lactate]	n-C ₄ H ₉					CH ₃ CH(OH)COO ⁻
[HMIm][PF ₆]	n-C ₆ H ₁₃					PF ₆ ⁻
[OMIm][BF ₄]	n-C ₈ H ₁₇					BF ₄ ⁻
[OMIm][PF ₆]	n-C ₈ H ₁₇					PF ₆ ⁻
[MOEMIm][BF ₄]	CH ₃ OCH ₂ CH ₂					BF ₄ ⁻
[PPMIm][PF ₆]	C ₆ H ₅ CH ₂ CH ₂ CH ₂					PF ₆ ⁻
1-Alkylpyridinium cations						
Abbreviation	R ¹				X ⁻	
[Epy][TFA]	H	C ₂ H ₅			CF ₃ COO ⁻	
[BMPy][BF ₄]	CH ₃	n-C ₄ H ₉			BF ₄ ⁻	
Alkylammonium cations						
Abbreviation	R ¹	R ²	R ³	R ⁴	X ⁻	
[EtNH ₃][NO ₃]	C ₂ H ₅	H	H	H	NO ₃ ⁻	
[Et ₃ MeN][MeSO ₄]	C ₂ H ₅	C ₂ H ₅	C ₂ H ₅	CH ₃	CH ₃ OSO ₃ ⁻	

(up to a molar fraction of 0.7) [19]. No ionic liquid dissolves in the CO₂ phase.

Thus, a theoretical basis for predicting the solvent properties of ionic liquids still has to be constructed. It has become clear, however, that ionic liquids do not fit into any of the standard heuristics that chemists traditionally use to assess and predict solvent behaviour. However, what can be predicted is that the characteristic property of some ionic liquids to mix neither with water nor with moderately non-polar organic solvents will revolutionise process design.

Ionic liquids and enzymes

The seminal work by Klibanov in the early 1980s [20,21] made it clear that enzymes can be used in hydrophobic organic solvents, although at the price of a severely reduced reaction rate [22]. It subsequently became clear that many lipases, as well as some proteases and acylases, are so stable that they maintain their activity even in anhydrous organic solvents. This characteristic is at the basis of the successful application of such hydrolases in non-hydrolytic reactions, such as the (enantioselective) acylation of alcohols and amines, which now are major industrial applications [23]. The desire to improve the low turnover rate of enzymes in organic media was a major driving force for extending the method to ionic liquids.

The complex interactions between electrolytes and proteins have been studied for more than a century [24,25]. However, understanding is not yet complete and

does not provide a basis for predicting the activity and stability of enzymes in ionic liquids. An early study of the alkaline phosphatase from *E. coli* in aqueous mixtures of [EtNH₃][NO₃], which is also the oldest ionic liquid on record [26], revealed an activating effect at low concentrations, reaching an optimum at 1.1 M (10%, v/v) [27]. The activity steeply decreased at higher concentrations but was recovered on dilution to 1.1 M. At 80% [EtNH₃][NO₃], however, all activity was irreversibly lost. Such a profile would not be unexpected for denaturing salts or organic solvents.

The first successful biotransformation in an ionic liquid medium containing 5% (v/v) water involved, perhaps not surprisingly, a hydrophobic ionic liquid. Thermolysin, a very stable enzyme, mediated the synthesis of Z-aspartame (see Fig. 1a) in buffer-saturated [BMIm][PF₆] medium at 40% of the turnover rate in ethyl acetate [28].

Lipases, noted for their tolerance of organic solvents, were obvious candidates for biocatalysis in ionic liquids. Indeed, stable microbial lipases, such as *Candida antarctica* lipase B (CaLB) [29–32] and *Pseudomonas cepacia* lipase (PcL) [31,33], were catalytically active in the ionic liquids of the 1-alkyl-3-methylimidazolium and 1-alkylpyridinium families, in combination with anions such as tetrafluoroborate, hexafluorophosphate and bis(trifluoromethanesulfonyl)imide. The early results were not always consistent, possibly due to impurities resulting from the preparation of the ionic liquid. Hence, a purification step is strongly recommended [16,33]. Lipases mediated transesterification (alcoholysis; Fig. 1b,c) reactions in these ionic liquids with an efficiency comparable with that in *tert*-butyl alcohol [29], dioxane [34] or toluene [33]. *Candida antarctica* lipase A, which was ten times more active in [BMPy][BF₄] and [BMIm][Tf₂N] than in diisopropyl ether [30], is an exception. The lipase from pig pancreas, the only mammalian lipase that has been subjected to ionic liquids, was inactive in transesterification [30,35].

Similarly, α-chymotrypsin mediated the transesterification (alcoholysis) of *N*-acetyl-L-amino acid esters (Fig. 1d) in ionic liquids of the 1-alkyl-3-methylimidazolium type [36–38], provided that the medium contained a small amount (~0.5%) of water. This requirement was lifted when the ionic liquids were combined with scCO₂ [36]. The transesterification rates in [BMIm][PF₆] and [OMIm][PF₆] medium were of the same magnitude as those in isoctane or acetonitrile [36] but in [EMIm][Tf₂N] (under slightly different reaction conditions) the rate was nearly an order of magnitude higher [37].

The lyophilisation of enzymes from solutions containing salts or amphiphilic compounds is known to increase the activity in organic media by up to several orders of magnitude. Thus, the transesterification activity of α-chymotrypsin was increased 82-fold by co-lyophilisation with pentaglyme [39]. Similar effects were noted for α-chymotrypsin in [OMIm][PF₆] medium, although the effect of (poly ethylene)glycol was less than in non-polar media [36]. The co-lyophilisation of the lipase from an unspecified *Pseudomonas* (PsL) and (poly ethylene)glycol increased the transesterification activity in [HMIm][PF₆] medium by a factor of five but the effect of the treatment on other lipases was very much less [40].

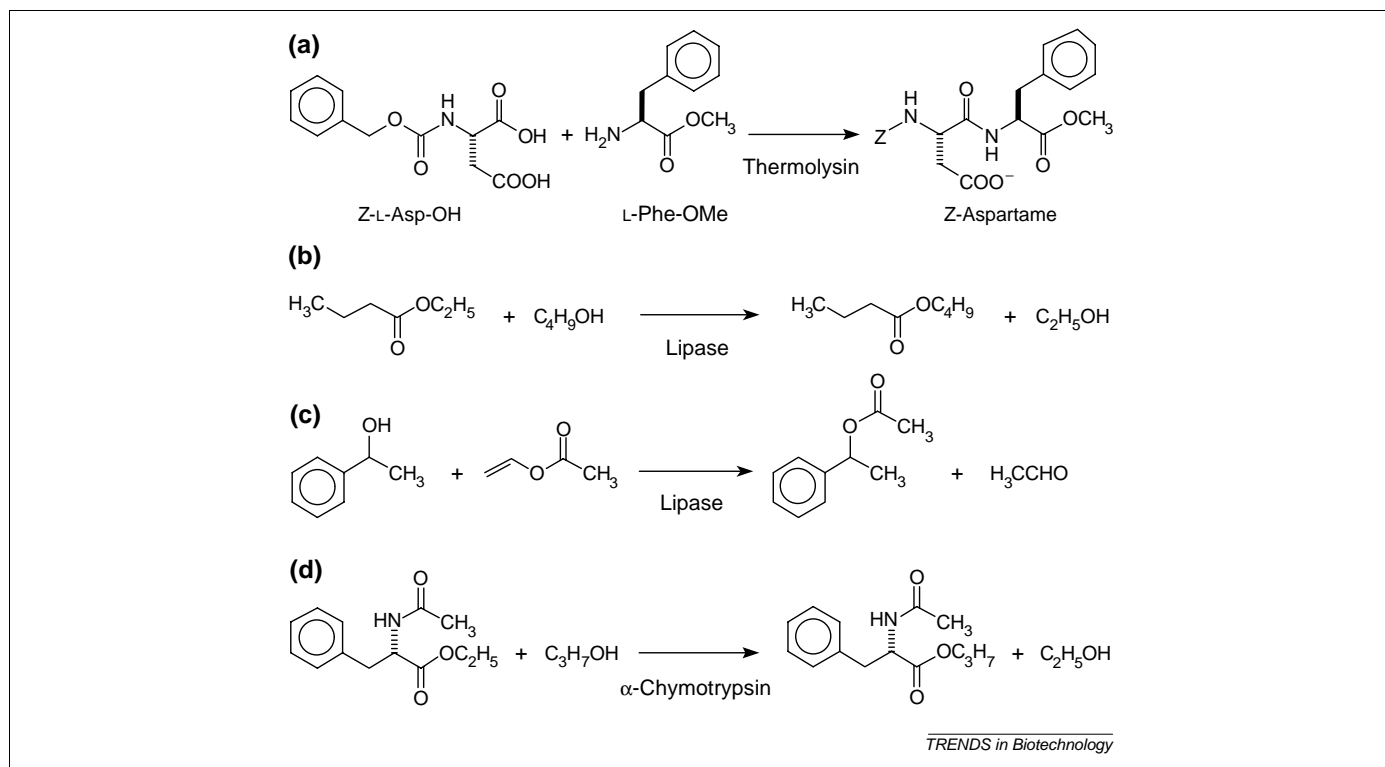


Fig. 1. Examples of enzymatic reactions performed in ionic liquid media.

The tolerance of lipases for ionic liquid media described was not universal. No reaction could be observed in very similar ionic liquids, such as [MMIm][MeSO₄] [30], [BMIm][NO₃], [BMIm][lactate] or [EtNH₃][NO₃] [7], all of which are water-miscible. It might be significant that the lipases dissolved in these media because dissolving a protein requires protein–protein interactions to be broken and be replaced by stronger ones. Water has this effect but the organic solvents, such as *N,N*-dimethylformamide and dimethylsulfoxide, which dissolve enzymes and, by implication, coordinate groups at the protein surface, also are strong denaturants. It is of interest that even the small amount (3.2 mg ml⁻¹) of thermolysin that dissolved in buffer-saturated [BMIm][PF₆] was not active [28]. We tentatively conclude that the methylsulfate, nitrate and lactate anions solvate the lipase and render it inactive. In support of this hypothesis, the denaturation of lysozyme on its dissolution in [EtNH₃][NO₃] has been observed using fluorescence spectroscopy [41].

The structural change that takes place when an enzyme dissolves in an ionic liquid is often reversible. Thus, when a solution of lysozyme in [EtNH₃][NO₃] was diluted with water the denatured enzyme recovered its full activity [41]. It was shown in separate experiments that 5% aqueous [EtNH₃][NO₃] acts as a renaturant on lysozyme. When CaLB was dissolved in [BMIm][NO₃], [BMIm][lactate], [BMIm][EtSO₄] or [EtNH₃][NO₃], allowed to stand for 24 h and diluted 50 times with buffer a substantial fraction of the original activity was recovered, ranging from 33% in [EtNH₃][NO₃] to 73% in [BMIm][NO₃] [7].

Glycosidases are sometimes used in aqueous-organic mixtures and therefore their tolerance for aqueous ionic liquids is of practical interest. The β-galactosidase

from *E. coli* had only 6% activity left in 50% aqueous [BMIm][BF₄] [32]. The β-galactosidase from *Bacillus circulans* was considerably more tolerant to ionic liquids; [MMIm][MeSO₄] was tolerated best (14% residual activity at 50% concentration [42]). The activity in pure [MMIm][MeSO₄] was very low but was completely recovered on dilution with water [42]. To put these observations into perspective, the residual activity of the *E. coli* enzyme in 50% aqueous ethanol and acetonitrile amounted to 7% and 3%, respectively [32].

Formate dehydrogenase tolerated aqueous ionic liquids much better than the galactosidases. The enzyme still had 98% activity left in buffer containing 75% [MMIm][MeSO₄] but [Et₃MeN][MeSO₄] was tolerated less (55% activity at 50% concentration) and [BMIm][TfO] still less (38% activity at 25% concentration) [42]. A range of ionic liquids – surprisingly including [BMIm][BF₄] – completely inhibited formate dehydrogenase even at 25% concentration [42]. These results contrast with the lesson learnt from the lipase work: that ionic liquids containing monoalkylsulfate ions are denaturants.

In summary, ionic liquids seem to affect enzymes in much the same way that conventional organic solvents do: some are tolerated well but others much less, depending on the nature of the enzyme.

The (thermal) stability (activity over time) of enzymes is often better in organic media, in particular at low water activity, than in aqueous medium. Ionic liquids can also have this effect. Thus, the activity loss of thermolysin proceeds much slower in [BMIm][PF₆] than in ethyl acetate [28] and the half-life of α-chymotrypsin in ionic liquids is ~10 times longer than in 1-propanol [37]. Lozano *et al.* [43] compared the stability of CaLB, in the presence of 2% of water, in a range of ionic liquids with that in

1-butanol or hexane and found that it was similar or better. Surprisingly, the life-time of CaLB increased by three orders of magnitude when substrate was present.

The thermal stability of CaLB, either free (Novozym SP525) or absorbed on a macroporous carrier (Novozym 435) also has been measured by incubating the preparation in anhydrous [BMIm][PF₆] at 80°C and measuring the residual activity, after dilution with water, in triacetin hydrolysis [7]. The activity of the free enzyme was found to increase in 20 h to 120% of an untreated sample, which was maintained for at least 100 h. By contrast, a linear deactivation versus time was observed in *tert*-butyl alcohol. The activity of Novozym 435 increased to 350% in 40 h, which on continued incubation slowly decreased to 210% after 120 h. By contrast, the incubation of a CLEC or CLEA [44] of CaLB in [BMIm][PF₆] at 80°C resulted in a progressive loss of activity, comparable with that observed in *tert*-butyl alcohol. It seems that the ionic liquid induces a more active conformation of the free enzyme, which evidently is not possible with the cross-linked preparations.

CaLB has a high operational stability at elevated temperatures in the absence of water. Novozym 435 retained its full transesterification activity in refluxing *tert*-butylalcohol for 7 days [45]. CaLB was exceptionally stable in (biphasic) [BMIm][Tf₂N]–scCO₂ systems, with an operational half-life of CaLB ranging from 400 h at 50°C to up to 60 h at 100°C [46].

Most studies of biocatalysis in ionic liquids have been concerned with the use of isolated enzymes. However, it should not be overlooked that the first report on biocatalysis and ionic liquids involved a whole cell preparation (*Rhodococcus* R312) in a biphasic [BMIm][PF₆]-water system [47]. It was later shown that baker's yeast [48], as well as *Rhodococcus* R312 and *E. coli* [49] maintain their activity in ionic liquids containing no or a very small separate aqueous phase. It seems that the ionic liquid is much less toxic to the cell membranes than a conventional organic solvent, such as toluene [47].

Biotransformations in ionic liquid medium

Lipases

The application of lipases in synthetic biotransformations encompasses a wide range of solvolytic reactions of the carboxyl group, such as esterification, transesterification (alcoholysis), perhydrolysis and aminolysis (amide synthesis) [50]. Transesterification and amide synthesis are preferably performed in anhydrous medium to suppress

unwanted hydrolytic side reactions. CaLB, which readily tolerates such conditions [51,52], as well as PsL and Pcl, are often used as the biocatalyst [53].

Lipase-catalysed transesterification to prepare polyesters (replacing the traditional chemical polymerisation at >200°C) has received considerable attention. CaLB mediates polyester synthesis in the ionic liquids [BMIm][BF₄] or [BMIm][PF₆] at 60°C [54] but the molecular weight of the product is low compared with a solventless system [55], perhaps owing to the high viscosity of ionic liquid media.

The lipase-mediated esterification of carbohydrates is hampered by the low solubility of carbohydrates in reaction media that support lipase catalysis in general. Because the monoacylated product (**1**, Fig. 2) is better soluble in traditional solvents than the starting compound the former tends to undergo further acylation into a diester (**2**). By contrast, the CaLB catalysed esterification of glucose in the ionic liquid [EMIm][BF₄] was completely selective. The reaction became much faster when conducted in [MOEMIm][BF₄] in which 5 g l⁻¹ of glucose dissolves at 55°C, 100 times more than in acetone [33]. The disaccharide maltose also was acylated in the presence of CaLB in [MOEMIm][BF₄]. Recently, up to 10 wt% of cellulose was dissolved in [BMIm][Cl] at 100°C [56], indicating that ionic liquids could possibly solve the incompatible demands of lipases and carbohydrates with regard to the reaction medium.

The resolution of chiral alcohols through lipase-mediated enantioselective acylation is one of the major industrial applications of lipases [23]. Hence, the effects of ionic liquid reaction media on the resolution of the arylalkanols **3–8** (Fig. 3) in the presence of, mainly, CaLB and Pcl have been investigated [30,31,33,35]. Vinyl acetate was universally adopted as the acyl donor. The alcohols discussed were, in general, resolved in traditional media with already good-to-excellent enantioselectivity; hence, there was little margin for improvement. Nevertheless, the enantiomeric ratio (*E*) [57] of some of these resolutions improved considerably when the reaction was performed in an ionic liquid. Thus, the enantiorecognition of **3** by PsL, which was only modest in *tert*-butyl methyl ether (TBME), became near-quantitative in [BMIm][TfO] or [BMIm][Tf₂N] [30]. Even the already excellent resolutions of **5–8** were improved in ionic liquid medium [31].

The high thermostability of lipases in ionic liquids has stimulated research into kinetic resolution at elevated

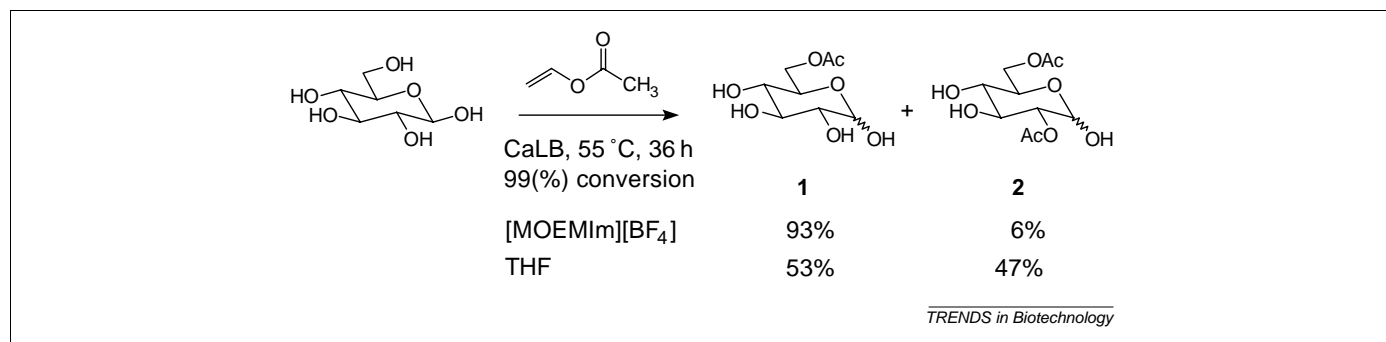


Fig. 2. The regioselective acylation of glucose in ionic liquid and traditional medium [33].

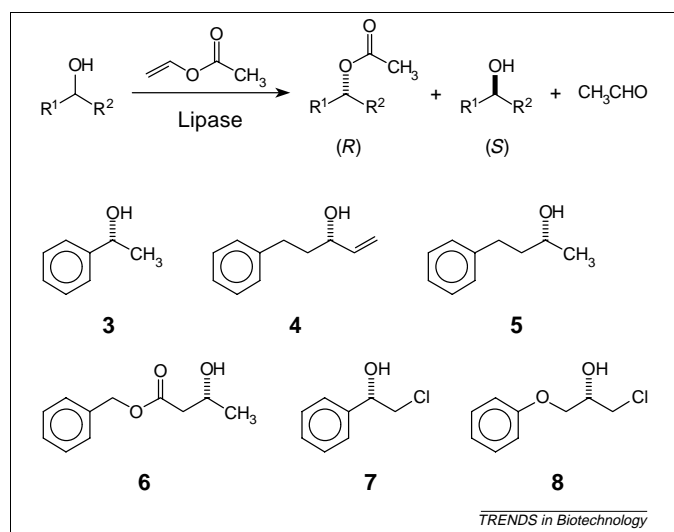


Fig. 3. The lipase-mediated kinetic resolution of chiral alcohols. The preferentially converted enantiomers have been drawn.

temperatures [58]. The PsL-mediated acylation of **3** by vinyl acetate in [BMIm][Tf₂N] remained highly enantioselective, *E* decreasing from 200 to 150, when the temperature was raised from 25 to 90°C. By contrast, the enantioselectivity in TBME medium dropped dramatically (from *E* = 200 to *E* = 4) at 55°C, which corresponds with the boiling point of TBME. In both solvents the decrease in *E* was observed at the boiling point of either the solvent (TBME) or vinyl acetate [58].

The resolution of phosphorus-substituted primary alcohols, such as **9**, in the presence of a *Pseudomonas fluorescens* lipase preparation (lipase AK) showed a remarkable dependence on the nature of the reaction medium [59]. The enantioselectivity in [BMIm][PF₆] was as good as, or better than, in diisopropyl ether (Fig. 4) but was negligible in [BMIm][BF₄]. (This effect cannot be ascribed to a contaminated ionic liquid because the authors stated to have purified the ionic liquids).

It has therefore become clear that the medium, either ionic liquid or traditional, has to be fine-tuned to the reactant and biocatalyst for optimum enantioselectivity. There is no 'best' ionic liquid for performing a kinetic resolution, just as there is no 'best' organic solvent in general and the theoretical basis for selecting one is still embryonic [60]. With the advent of ionic liquids the choice of solvents and thus the chance to find one that is satisfactory has increased enormously.

Lipases mediate a variety of non-natural reactions but such examples in ionic liquid medium still are scarce and

restricted to perhydrolysis and ammoniolysis (Fig. 5a,b). Perhydrolysis has been demonstrated, amongst others, in the epoxidation of cyclohexene by peroctanoic acid that had been generated *in-situ* from octanoic acid and hydrogen peroxide in the presence of CaLB (Fig. 5b) [61]. In [BMIm][BF₄] the reaction rate was slightly lower than in acetonitrile [29], which is the optimum solvent for this reaction (De Zoete, M.C., Ph.D. Thesis, Delft University of Technology, 1995). The reaction of ethyl octanoate or octanoic acid with ammonia, to give octanamide, was catalysed by CaLB in [BMIm][BF₄]; the ester was converted at 40–70% of the rate in *tert*-butyl alcohol, depending on the formulation of the biocatalyst [29].

Protease

Subtilisin is an endoprotease that has been used in the enantioselective hydrolysis of *N*-acylamino acid esters (**11**, Fig. 6) into the corresponding (*S*)-amino acids (**12**). An organic solvent is often added to improve the solubility of the amino acid derivative. The reaction became more enantioselective when it was carried out in [EPy][TFA]-water (15:85) instead of acetonitrile-water (15:85) [62].

Glycosidases

In their natural role, glycosidases hydrolyse glycosidic bonds but they are also widely used as biocatalysts for carbohydrate synthesis *in vitro*, such as in the transesterification of lactose with *N*-acetylglucosamine. The competing secondary hydrolysis of the product, *N*-acetylglucosamine limits the yield. This secondary hydrolysis could be suppressed by performing the reaction in [MMIm][MeSO₄]-water (25:75, v/v), with an increase in product yield from 30% in aqueous buffer to 58% in aqueous ionic liquid [42].

Redox enzyme systems

Biocatalytic redox reactions are often carried out using whole-cell biocatalysts, owing to the necessity of recycling the redox cofactor. The organic phase, which is often used to store the sparingly soluble reactants and products, can be replaced by an ionic liquid, which is less harmful to the cell membranes [49]. Thus, a range of ketones was enantioselectively reduced by an immobilised yeast in a [BMIm][PF₆]-water (10:1) biphasic medium [48]. The performance of the system was, on average, comparable with that in a conventional aqueous–organic medium.

Reaction systems

We have only discussed the straightforward use of ionic liquids as reaction medium, either as such or as a

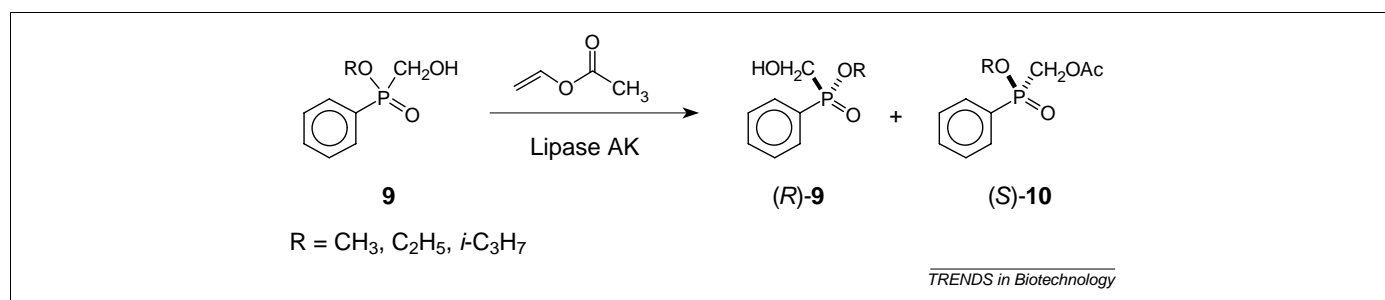


Fig. 4. The enantioselective acylation of phosphate-substituted primary alcohols [59].

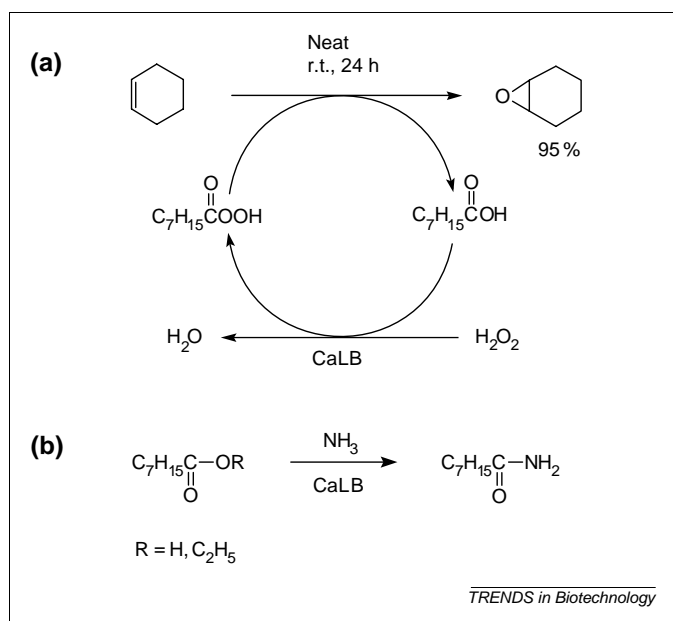


Fig. 5. Perhydrolysis and ammoniolysis mediated by CaLB in ionic liquid medium [29].

monophasic aqueous mixture. Even then, the unique properties of ionic liquids allow the use of unconventional reaction techniques.

Catalyst recycling

As previously mentioned, ionic liquids, such as [BMIm][PF₆], do not mix with ether. This unconventional behaviour was advantageously used by extracting the products and the unconverted reactant from the transesterification mixture of **4** (Fig. 3) with ether. The lipase biocatalyst remained suspended in the ionic liquid phase and could be recycled [35]. An observed loss of activity was ascribed to the accumulation of inhibiting acetaldehyde oligomers in the ionic liquid phase [63].

PcL has been entrapped in an ionic solid through dispersion in the relatively high-melting ionic liquid [PPMIm][PF₆], which was allowed to solidify and broken into small particles [64]. The resulting biocatalyst could be reused at least five times in transesterification.

Product evaporation

Because ionic liquids lack a vapour pressure, products can be removed by evaporation, as was shown in the reduction of prochiral ketones by baker's yeast in [BMIm][PF₆] [48]. Evaporation of the alcohol side-product in lipase-catalysed transesterification reactions can be used to drive the

equilibrium towards complete conversion. Thus, in the transesterification of **4** the usual vinyl ester can be replaced by a methyl ester. Because the procedure does not liberate inhibiting acetaldehyde the biocatalyst could be recycled without deactivation [63].

Two-phase system with supercritical CO₂

The above-mentioned approach of retaining the biocatalyst in the ionic liquid reaction medium has been further developed into a biphasic reaction system. The enzyme is retained in an ionic liquid working phase and the reactants and products largely reside in a scCO₂ extractive phase [46,65]. The principle has been shown with CaLB in simple model transesterifications as well as in the enantioselective acylation of **3** in batchwise and continuous procedures; vinyl esters were used as the acyl donor. The high operational stability of CaLB, which contrasts with the generally rapid deactivation in pure scCO₂, is one of the attractive aspects of this approach. The reaction rate was approximately eight times better than that in pure scCO₂ under otherwise identical conditions [46].

Two-phase aqueous systems

Two-phase aqueous reaction systems, consisting of an aqueous working phase and an organic extractive phase, are widely used in biotransformations of hydrophobic compounds. They are useful with biocatalysts that require an aqueous phase for activity, in particular when water does not interfere with the desired reaction, as is the case, for example, with non-hydrolase biocatalysts. The replacement of organic solvents as the extractive phase by the hydrophobic ionic liquid [BMIm][PF₆] has, until now, only been demonstrated with whole-cell nitrile hydratase-amidase [47] and redox biocatalysts [48,49], as well as in the recovery of butanol from acetone–butanol-ethanol fermentations [66]. The technique could also prove to be useful with isolated enzymes, provided that the deactivation at aqueous–organic interfaces to which many enzymes are prone can be obviated by proper design of the ionic liquid.

Conclusion

Hopefully this review has shown that a variety of enzymes, particularly those that tolerate conventional organic solvents, are eminently capable of performing in ionic liquids. Activities are generally comparable with or higher than those observed in conventional organic solvents.

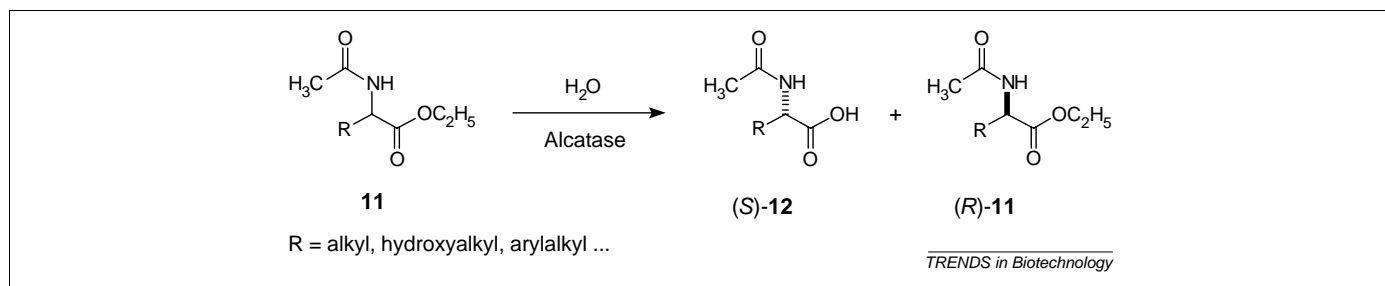


Fig. 6. Enantioselective hydrolysis of *N*-acetylamino acid esters [62].

Furthermore, enhanced thermal and operational stabilities and regio- or enantioselectivities have been observed.

Ionic liquids have obvious potential as reaction media for certain biotransformations of highly polar substrates, such as (poly)saccharides, amino acids and nucleotides, which cannot be performed in water owing to equilibrium limitations. An important challenge is to use the unique solvent properties of ionic liquids to develop efficient methods for product separation and ionic liquid recycling. The development of less expensive ionic liquids will further stimulate their use in industrial biotransformations. It will be interesting to ascertain if enzymes can be modified to render them active when dissolved in ionic liquids, analogous to the stabilization of enzymes dissolved in organic solvents, for example as (poly ethylene)glycol conjugates. In short, we believe that biotransformations in ionic liquids hold much promise for the future.

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