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INVITED REVIEW PAPER

Enzyme performance in ionic liquids

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Abstract–Ionic liquids (ILs) having unique properties such as no measurable vapor pressure, nonflammability and a wide temperature range of liquid phase have been recognized as potential green solvents. As a result, ILs have been extensively explored as reaction media for various biocatalytic reactions over a decade. Enzyme activities in ILs are generally comparable with or higher than those observed in conventional organic solvents. Furthermore, enhanced thermal and operational stabilities and regio- or enantioselectivities have been observed in many cases. Thus, ILs offer new possibilities for the application of solvent engineering to biocatalytic reactions. This review discusses the effect of physicochemical properties of ILs on biocatalysis with respect to enzyme activity, stability and selectivity by systematizing literature data on enzyme-catalyzed reaction in ILs.

Key words: Ionic Liquids, Biocatalysis, Enzyme Activity, Enzyme Stability, Physicochemical Properties

INTRODUCTION

Ionic liquids (ILs) are not new, some of them have been known for many decades. For instance $[EtNH_3][NO_3]$, which has a melting point of 12 °C, was first described in 1914 [1]. The interest in these compounds, often regarded as the green, high-tech media of the future, is still increasing rapidly and stems from their near-zero vapor pressure, their thermal stability and their widely tunable properties as regards to polarity, hydrophobicity, and solvent miscibility behavior through appropriate modification of the cation and the anion [2-4]. The structures of commonly used cations and anions of ILs were shown in Fig. 1. Based on their renowned tunable properties, they show an increasing potential to revolutionize reaction technology. Their synthesis, physicochemical properties, and major fields of application have been reviewed [5] and the number of applications of ILs as reaction media for organic synthesis and catalysis is growing rapidly.

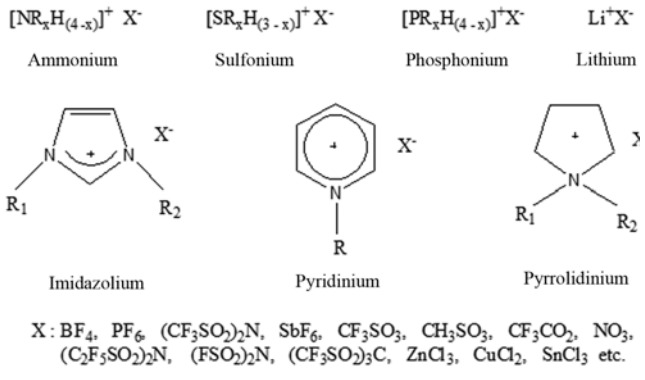

Fig. 1. Structures of ILs commonly used for biocatalytic reactions.

The use of ILs in biocatalysis was preceded by studies on enzymatic reactions in organic solvents [6-8]. The discovery of efficient biocatalysis in non-aqueous polar [6-8] and nonpolar media [9,10] and the unique physicochemical properties of ILs encouraged studies on the replacement of molecular organic solvents with ILs in biochemical processes. The first study on the use of ILs in biocatalysis was devoted to the yeast cell *Rhodococcus* R312 in the two phase water-$[Bmim][PF_6]$ system [11]. Furthermore, it was found that not only *Rhodococcus* R312 but also *E. coli* retained their biological activity in a number of ILs containing no water or a small amount of water. This fact was indirect evidence of the environmental safety of ILs unlike traditional organic solvents. In 1984, one study found an activating effect of $[EtNH_3][NO_3]$ on the catalytic activity of alkaline phosphatase from *E. coli* in the oxidation reaction of *p*-nitrophenyl phosphate at an ILs concentration of 1.1 M [12].

At the present time, there have been several excellent reviews that focus on enzyme-catalyzed reactions in ILs [13-17]. Among these, an excellent review published by Y. H. Moon et al. has highlighted the enzyme-catalyzed reactions in ILs including transesterification, synthesis, conversion, ammonialysis, hydrolysis, epoxidation, resolution and oxidation [13]. More recently, van Rantwijk and Sheldon have published a well balanced and comprehensive review regarding the biocatalysis in ILs where the effects of ILs on the structure and activity of enzymes as well as on their thermal and operational stability were discussed [17].

The use of ILs as reaction media is greatly expanding the range of enzyme-catalyzed reaction. Studies over the past decade have established firmly that many enzymes can work in ILs containing small amount of water or no water [18,19]. Additionally, the performances of enzymes in ILs are improved significantly by immobilization or modification with solid supports [20,21]. These trends are now emerging by discovering new, unique and useful techniques in which enzymes exhibit more pronounced activity, stability as well as selectivity in ILs.

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In this review, we will discuss the effects of ILs on enzyme performance with respect to enzyme activity, stability and selectivity, and their applications in biocatalysis with an emphasis on the accessible literature published in this field of research over a decade. This will provide various issues on enzyme-catalyzed reactions studied in ILs or IL-based solvent systems and its application to bioprocesses.

COMMON PROBLEMS ARISING FROM THE USE OF MOLECULAR ORGANIC SOLVENTS IN BIOCATALYSIS

In the past few years, there are several limitations on the use of organic solvents in enzymatic catalysis that has been discovered. Some of the most common limitations that are experienced in using conventional organic solvents are discussed in the following:

1) A change from water to an organic solvent frequently results in either complete enzyme denaturation or a dramatic decrease in enzyme catalytic activity and stability and a change or disappearance of substrate specificity [22].

2) Organic solvents affect contacts between the participants of an enzymatic reaction or change the solubility of the substrate and its distribution in the system [23].

3) Proteins can dissolve in polar organic solvents miscible with water, such as acetone, acetonitrile, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO), but they lost their activities in the majority of cases if the organic solvent concentration was ≥30-70 vol% [24].

4) High polarity solvents can absorb water and remove a hydrate shell from an enzyme to cause loss in its catalytic activity [25].

5) Although proteins are insoluble in nonpolar hydrophobic solvents such as heptane, octane, and benzene, they are inert toward enzymes because of the spatial separation of system components and they do not cause the dehydration of biocatalysts [23].

6) Hydrophilic proteins in almost anhydrous nonpolar solvents form suspensions, whereas proteins with extended hydrophobic surface segments and lipoproteins can form microemulsions in the same media [24,26].

The problems listed above are indeed important considerations in biocatalytic processes in the presence of molecular organic that needed to be address. The seminal works by Klibanov in the early 1980s made it clear that enzymes can be used in hydrophobic organic solvents, but at the price of a severely reduced reaction rate [27, 28]. The general desire to improve the low turnover rate of enzymes in organic media was a major driving force for extending the method to ILs. In the following section, therefore, the use of ILs as an alternative molecular solvent will be discussed together with the justification of these substances in each application.

IONIC LIQUIDS AS ALTERNATIVE MOLECULAR SOLVENTS

Due to prevailing disadvantages found in using molecular organic solvents, it opens the opportunity for ILs as an alternative solution and in fact it provides superior results as compared to organic solvents which were already proven by several studies. It has been proven that in addition to enhanced reaction rates and conversion, improved selectivity and regioselectivity were also observed with use of ILs [18,29]. Among numerous advantages of ILs which can offer, the broad temperature range of a liquid state of ILs from −80 to 350 °C is considered to be of paramount importance in chemical technology in general and in biotechnology because most of the biochemical processes are performed at elevated temperatures. Furthermore, the range of ILs depends on the number of possible combinations of anions and cations and the introduction of various substituents into the cations. This is an additional chemical instrument for controlling the physicochemical properties of ILs such as melting temperature, density, viscosity, polarity, and miscibility with water or organic solvents, depending on the scientific problem to be solved. Extensive experimental data on the physicochemical properties of ILs were obtained and successful experience in the use of ILs in inorganic, organic, and electrochemical syntheses and catalysis was accumulated [30-32]. In the present section, therefore, the physicochemical properties of ILs that justifies their advantages over organic solvents in biocatalytic reactions will be discussed.

1. Hydrophobicity and Polarity

One of the notable properties of ILs is that they are capable of a wider range of intermolecular interactions such as dipolar, hydrogen bonding, dispersive and ionic [33,34]. Hence, many compounds are significantly soluble in ILs. Interestingly, ILs having coordinating anions (e.g., Cl^-, NO_3^-, CH_3COO^- and $(MeO)_2PO_2^-$) which are strong hydrogen bond acceptors can dissolve many compounds which are insoluble or sparingly soluble in water and most organic solvents. Examples include carbohydrates [35] cellulose [36,37], amino acid [38,39] and some compounds having pharmacological activity [40, 41]. The ability of ILs to dissolve such compounds generally depends on the hydrogen bonding ability of anions [33,37]. Moreover, the immiscibility of ILs with either water or organic solvents has made them feasible to be used to form a two-phase system. Last but not the least is that the polarity of ILs can affect enzyme stability and selectivity [18,19,29,42]. In general, polar solvents increase the solubility of polar substrates and lead to faster and more selective reactions [19,43].

2. Stability

ILs are nonexplosive since they have a minimum vapor pressure. Their decomposition temperature varies from 150 to 450 °C, depending on the nature of the cations and anions; that is, their thermal stability is relatively high. ILs based on imidazolium cations are most stable [44,45]. Therefore, they are very effective as solvents for high temperature reaction systems.

3. Viscosity

The dynamic viscosity (μ) of ILs is higher than the viscosity of water at room temperature by a factor of tens or hundreds. The introduction of bulky substituent and the elongation and branching of alkyl chains in the cation structure considerably increase the viscosity of ILs. Furthermore, the viscosity of ILs was affected more by the change to the anion than to the cation [46]. The solvent viscosity could affect the biocatalytic reaction rates in terms of the mass transfer limitation when the reaction is rapid. Generally, high viscosities of solvents can be translated into good stabilization for enzymes and this was argued by van Rantwijk and Sheldon wherein the change of enzymes conformation is slow in high viscous ILs, which is effective for maintaining activity and stability of enzymes for long period [17]. Due to this property of ILs which shows an excellent

stability of enzymes to elevated temperatures in high viscous ILs it open up the possibilities of biocatalytic reactions with several different incubation protocols.

4. Biocatalyst Recycling and Product Recovery Schemes

This last advantage of ILs is technically not a physicochemical property but its importance in ILs as a reaction media for biocatalysis makes it worth mentioning in this section. Having this characteristics found in ILs it validates the pronounced advantage of ILs over molecular organic solvent since biocatalyst recycling and product recovery schemes are both not feasible with traditional organic solvent systems. As previously mentioned, most of ILs do not mix with organic solvents such as hexane and ether. This unconventional solvent property allowed extraction of the product and unconverted reactant simply just washing with diethyl ether and hexane or even supercritical CO_2 [47,48]. In this case, the biocatalyst remained in the IL phase and can be recycled for further use [49-51]. Hence, the resulting biocatalyst could be reused at least 4-5 times without loss of their activity. Furthermore, in some cases the products can be simply separated by evaporation due to the lack of vapor pressure of ILs [52].

MAJOR FACTORS AFFECTING ENZYME ACTIVITY AND STABILITY IN ILs

In order to determine the enzyme-stabilizing or activating methods, it is important to know what physical properties are most important to the catalytic behaviors of enzymes in ILs. It is well known that an enzyme's performance in ILs is affected by common factors such as the water activity, pH, excipients and impurities [16]. In addition, the solvent properties of ILs are often related to the enzyme's activity, specificity and stability.

1. IL Polarity

Based on the solvatochromic studies, ILs were found to be moderately polar being close to lower alcohols [53,54] or formamide [55]. Park and Kazlauskas found that the trend of lipase (from *Pseudomonas cepacia*) activity was increasing with the IL polarity during the acetylation of racemic 1-phenylethanol with vinyl acetate [29]. In another investigation, lower synthetic activities of α-chymotrypsin were also obtained in less polar ILs [19]. During Novozym 435-catalyzed esterification of methyl-α-D-glucopyranoside with fatty acids, Mutschler et al. observed that the ester conversion increased with IL polarity when ILs were used as liquid film-coating (under solvent-free conditions), but decreased with IL polarity when ILs were used as solvents [56]. However, the IL polarity-enzyme activity correlation has not been established for other enzymatic reactions performed in ILs [18,42,57].

2. Hydrogen-bond (H-bond) Basicity and Nucleophilicity of Anions

These are the two different concepts, but are often closely related. For molecules containing the same nucleophilic atoms of the same charge, the stronger base is usually the stronger nucleophile in aprotic solvents.

[Bmim][Cl] could effectively dissolve cellulose because chloride ions as H-acceptors interact with the cellulose -OH group and break the H-bonding network of cellulose [58,59]. As a result, this IL induced the inactivation of *Trichoderma reesei* cellulase (from *Trichoderma reesei*) [60]. Correspondingly, Lee et al. also observed a dramatic decrease of the lipase activity in [Omim][Tf_2N] with the increasing concentration of [Omim][Cl] [61]. Based upon multiple solvation interactions, [Bmim][Cl] showed the largest H-bond basicity among all ILs considered in the study by Anderson et al. [33], and thus could dissolve complex polar molecules such as cyclodextrins and antibiotics [62]. Lou et al. reported that Novozym 435 showed no ammonolysis activity towards (*R,S*)-*p*-hydroxyphenylglycine methyl ester in [Bmim][Br] and [Bmim][NO_3], implying the denaturing nature of these two ILs [63]. Lau et al. suggested that the low CALB (lipase B from *Candida antarctica*) activity in [Bmim][lactate] was caused by the secondary structure changes of the protein, which was further triggered by the H-bonding interaction between lactate anions and peptide chains [64]. Dicyanamide (dca) based ILs such as [Bmim][dca] are able to dissolve carbohydrates [65,66], but [Bmim][dca] works an enzyme denaturing IL possibly due to the H-bond basicity of the anion [67-69]. Zhao et al. also suggested both free and immobilized CALB in [Emim][TfO] were about as active as in [Bmim][dca], which were less active than in other ILs [70].

The free *Candida rugosa* lipase was determined that it is only active in hydrophobic [Bmim][PF_6], but inactive in all hydrophilic ILs based on NO_3^-, CH_3COO^- and CF_3COO^- during the transesterification of methylmethacrylate with 2-ethyl-1-hexanol [42]. It was indicated that the latter three anions are more nucleophilic than PF_6^-, and thus could interact with the enzyme causing the protein conformational changes. Hernandez-Fernandez et al. reported the stability of CALB in ILs to be in the following order: [Hmim][PF_6]>[Hmim][Tf_2N]>[Hmim][BF_4], and [Bmim][PF_6]>[Bmim][dca], and the stability of Penicillin G acylase was in a similar order [Bmim][Tf_2N]>[Bmim][PF_6]>[Bmim][BF_4] [71]. They explained that the decreasing stability was, in general, consistent with the increasing order of nucleophilicity ($PF_6^- < BF_4^- < Tf_2N^- < dca^-$), where the more nucleophilic anions tend to interact with the positively charged sites in enzymes and to modify the enzyme's conformation. Alternatively, they also realized that the enzyme stability was in agreement with the hydrophobicity of ILs: both enzymes were more stable in hydrophobic ILs than in hydrophilic ones. However, in another study, a contradictory result was reported. Irimescu and Kato carried out the CALB-catalyzed enantioselective acylation of 1-phenylethylamine with 4-pentenoic acid, and found that the reaction rate relied on the type of IL anions (reaction rate in a decreasing order of $TfO^- > BF_4^- > PF_6^-$ for the same cations) [72]. This suggests higher anion nucleophilicity correlating with higher enzymatic activity. In a second acylation reaction of 2-phenyl-1-propylamine with 4-pentenoic acid, however, they observed that PF_6^- based ILs afforded fastest reaction rates, followed by TfO^- and BF_4^- based ILs. The rather confusing results may be because the enzymatic reaction is affected by multiple factors of ILs such as nucleophilicity, hydrophobicity, viscosity and impurity. Lee et al. measured the initial transesterification rates of three lipases (Novozym 435, *Rhizomucor miehei* lipase, and *Candida rugosa* lipase) in different ILs under the same water activity (a_w), and observed that the anion effect on the initial rates followed a decreasing order $Tf_2N^- > PF_6^- > TfO^- > SbF_6^- \sim BF_4^-$ [73]. They explained that TfO^- and BF_4^- are more nucleophilic than PF_6^-. The second factor could be IL hydrophobicity because lipases seemed more active in hydrophobic ILs than in hydrophilic ones.

3. Ion Kosmotropicity

In aqueous solutions, hydrophilic ILs dissociate into individual ions.

Therefore, recent studies proposed that ion kosmotropicity (Hofmeister series) is important in interpreting the enzyme's behaviors in aqueous IL solutions: kosmotropic anions and chaotropic cations stabilize the enzyme, while chaotropic anions and kosmotropic cations destabilize it [57,74-76]. Recently, Fujita et al. correlated the activity of cytochrome *c* in ILs containing 20 wt% water with the kosmotropicity of component ions [77]. Constantinescu et al. also confirmed that the thermal stability of ribonuclease A (RNase A) in aqueous solution of ILs follows the Hofmeister series [78]. Several studies reported low or no activities of β-glycosidase in aqueous solutions of [Bmim][BF_4], which may be explained by the chaotropic nature of BF_4^- in solutions [79,80]. An excellent review by Yang systematically discussed the possible mechanisms of Hofmeister effects of ILs on the enzyme activity and stability [81]. The above preliminary studies have shown that the kosmotropic effect of ILs on enzymes may be applicable to diluted aqueous solutions of ILs [57,74,75], as well as some concentrated ILs (such as 20 wt% water) [77]. However, it is not quite clear if such an effect exists in pure ILs or ILs with trace amounts of water, and how the IL hydrophobicity may influence the kosmotropicity. For example, PF_6^- is a chaotropic anion [76] and denatures enzymes when dissolved in aqueous solutions as Na^+ or K^+ salt (more denaturing than BF_4^- and $MeSO_4^-$ for mushroom tyrosinase) [82]. However, PF_6^- based ILs (such as [Bmim][PF_6]) are hydrophobic, and thus the solubility and degree of ion dissociation of ILs in water are limited. On the other hand, it is also known that PF_6^- based ILs are typically enzyme stabilizing [17]. Therefore, the Hofmeister effect may not be suitable for explaining the enzyme's behavior in these hydrophobic ILs or their mixtures with water.

4. Hydrophobicity

This may be considered as a narrower concept of 'polarity'. However, it is practically important to separate 'hydrophobicity' from 'polarity' because the former is often related to the miscibility with water [83]. The hydrophobicity of ILs is usually quantified by the log *P* scale, a concept derived from the partition coefficient of ILs between 1-octanol and water. Russell et al. measured the log *P* values (<-2.0) of several ILs [42], and suggested that they are very hydrophilic in nature based on Laane's scale [83-85]. They also observed that free *Candida rugosa* lipase was only active in hydrophobic [Bmim][PF_6] (log $P=-2.39$), but inactive in other hydrophilic ILs including [Bmim][CH_3COO] (log $P=-2.77$), [Bmim][NO_3] (log $P=-2.90$) and [Bmim][CF_3COO]. Likewise, Nara et al. achieved higher transesterification activities of lipases in [Bmim][PF_6] than in [Bmim][BF_4] [86]. Goto et al. also reported higher activities of PEG-modified lipase and subtilisin in more hydrophobic ILs such as [Emim][Tf_2N] [87,88]. Zhang et al. reported low penicillin acylase stabilities in [Bmim][BF_4] and [Bmim][dca] [89]. Lou and Zong studied the enantioselective acylation of (*R*,*S*)-1-trimethylsilylethanol with vinyl acetate catalyzed by lipases in several ILs, and indicated that the activity, enantioselectivity and thermostability of Novozym 435 increased with IL hydrophobicity in the order ([Bmim][PF_6]>[Omim][BF_4]>[C_7mim][BF_4]>[Hmim][BF_4]>[C_5mim][BF_4]>[Bmim][BF_4]) [90]. Paljevac et al. reported that cellulase activity decreased in the order of IL hydrophobicity: [Bmim][PF_6]>[Bmim][BF_4]>[Bmim][Cl] [96]. In other study, it was observed that penicillin G acylase shown a lower stability in [Bmim][BF_4] than in hydrophobic ILs (Tf_2N^- and PF_6^-), particularly in the absence of substrate [92]. Recent study on the alcoholysis of vinyl butyrate and 1-butanol by free CALB suggested that the lipase activities were generally much lower in water-miscible ILs (such as BF_4^-, dca^-, NO_3^-, CH_3COO^-, etc.) than in water-immiscible ones (PF_6^- and Tf_2N^-), and the enzyme's activities increased with the cation's hydrophobicity ($Emim^+ < Bmim^+ < Hmim^+ < Omim^+$) [93]. Ha et al. also found Novozym 435 was less active and stable in [Bmim][BF_4] than in other hydrophobic ILs. Lee et al. reported that Novozym 435 was more thermally stable in hydrophobic ILs than in hydrophilic ones following the order [Bmim][Tf_2N]>[Bmim][PF_6]>[Bmim][TfO]>[Bmim][BF_4]>[Bmim][SbF_6] [73]. Shen et al. noticed that during the kinetic resolution of racemic cyanohydrins, Amano lipase PS showed a high enantioselectivity (80% ee_p) in hydrophobic [Omim][PF_6], but poor enantioselectivities (<5% ee_p) in hydrophilic [Hmim][BF_4] and [Hmim][Cl] [95]. One study concluded that both free CALB and penicillin G acylase (PGA) are more stable in hydrophobic ILs than in hydrophilic ones: in the case of CALB, the stability is in decreasing order [Hmim][PF_6]>[Hmim][Tf_2N]>[Hmim][BF_4], [Bmim][PF_6]>[Bmim][dca], and [Omim][PF_6]>[Hmim][PF_6]>[Bmim][PF_6]; in the case of PGA, the stability is in a decreasing order of [Bmim][Tf_2N]>[Bmim][PF_6]>[Bmim][BF_4] [71]. However, the hydrophobic cations showed an adverse effect on PGA stability: [Emim][Tf_2N]>[Bmim][Tf_2N], and [Bmim][PF_6]>[Omim][PF_6]. Through a systematic investigation of lipase-catalyzed transesterification in over 20 ILs, it was observed that the lipase activity increased with the log *P* value to a maximum, and then decreased with further increase in log *P* like a bell shape [69]. These examples implied that the high hydrophobicity (large log *P*) of ILs could be beneficial to enzyme stabilization. However, a higher log *P* of the solvent also means a more thermodynamic ground state stabilization of substrates resulting in lower conversion of the substrate [96]. This could explain the decreasing reaction rate in very hydrophobic ILs. Similarly, Lou et al. found that the initial rates of Novozym 435-catalyzed ammonolysis of (*R*,*S*)-*p*-hydroxyphenyl glycine methyl ester increased with the hydrophobicity of BF_4 based ILs to a maximum, and then declined with further increase in hydrophobicity [63]. Conflictingly, some studies reported relatively high enzyme activities in hydrophilic ILs (such as [Bmim][BF_4], [Emim][BF_4] and [Bmim][TfO]) [29,97-100]. Therefore, multiple factors must be considered in explaining the enzymatic systems like these.

5. Viscosity

ILs are more viscous fluids than conventional organic solvents and many enzymatic reactions in ILs are heterogeneous due to the low solubility of enzymes in most pure ILs. Therefore, internal and external mass-transfer limitations should be considered. Lozano et al. indicated that besides the IL polarity, the activity of α-chymotrypsin also depended on the IL viscosity [19]. Higher enzyme activity was observed in [Emim][Tf_2N] than in [MTOA][Tf_2N] because the former IL (34 mPa) is less viscous than the latter one (574 mPa). Eckstein et al. explained that the higher enantioselectivity of lipase in [Bmim][Tf_2N] at low water activities ($a_w<0.53$) than in MTBE was for two reasons: (a) the higher viscosity of IL (52 mPa) than that of MTBE (0.34 mPa) may lead to mass transfer limitations and lower the reaction rate; (b) the lower solubility of substrates in the IL than in MTBE may cause a lower activation energy in the IL [101]. Van Rantwijk et al. explained that the high viscosity of ILs slows down the conformational changes of proteins, allowing enzymes

to maintain their native structures and activity [83]. However, Basso et al. observed that during amide synthesis through immobilized penicillin G amidase, the viscosities of [Bmim][PF_6] and [Bmim][BF_4] did not affect the initial reaction rates despite their much higher viscosities than toluene [102]. A recent study of the lipase-catalyzed transesterification of ethyl butyrate and 1-butanol in more than 20 ILs further confirmed that the IL viscosity might affect the reaction rates in some cases, but is not the primary factor in controlling the enzyme's activity [69].

6. IL Network

ILs can form so-called organized 'nano-structures' (hydrogen bonded polymeric supramolecules, which are similar to water molecules) with polar and non-polar regions in solid, liquid and solution states, or even in the gas phase [103,104]. Dupont suggested that the aqueous solution of free enzymes might be embedded in the IL network, which could protect the essential water of proteins and the solvophobic interactions that are critical for maintaining the native structure of proteins [104]. However, since enzymes are not soluble in most common ILs, enzyme molecules are suspended in reaction media at low water contents (for example, CALB is known to be active in the absence of water) [69,105]. As a result, IL network theory is not appropriate for understanding these enzymatic reactions.

7. Enzyme Dissolution

Hydrophobic ILs (typically consist of PF_6^- and Tf_2N^-) do not dissolve appreciable amounts of enzymes. On the other hand, hydrophilic ILs (such as those based on NO_3^-, lactate, $EtSO_4^-$, and CH_3COO^-) may dissolve some enzymes, however, most of them tend to strongly interact with proteins (such as via H-bonding), resulting in enzyme inactivation [60,64,67-69,93]. For example, cellulose was dissolved but inactivated in concentrated solutions of [Bmim][Cl] [60]. Currently, there are only a few pure ILs that are known to dissolve enzymes but do not inactivate them. For example, choline dihydrogen phosphate (m.p. 119 °C) containing 20 wt% water was found to solubilize and stabilize cytochrome *c* (cyt *c*) [70] and [Et_3MeN][$MeSO_4$] was reported to be able to dissolve>1.2 mg/mL CALB and retain its catalytic capability [64,76]. Recently Zhao et al. synthesized a series of ether-functionalized ILs that are able to dissolve enzymes (>5 mg/mL CALB at 50 °C) and a variety of substrates, but do not inactivate the lipase (more discussion in a later section) [67,70]. Therefore, the enzyme dissolution in ILs is not a definite indication of enzyme denaturation.

8. Surfactant Effect

ILs bearing long alkyl chains in their cations often form self assembly in aqueous solutions and behave like surfactants [105,106]. Ionic surfactants, such as sodium *n*-dodecyl sulfate (SDS) and *n*-dodecyltrimethylammonium bromide (DTABr), interact with enzymes through two major mechanisms: (a) electrostatic interactions of the surfactant head group and charged amino acid residues of the protein, and (b) hydrophobic interactions between the alkyl chains of the surfactant and hydrophobic amino acid residues [107]. In general, ionic surfactants are considered non-specific denaturants of enzymes, but many studies also reported that they either have no effect on enzymes, or show some activating or stabilizing effects on enzymes [107-109]. The knowledge of surfactant-protein interactions can be useful for understanding the IL effect on enzyme activity and stability in some systems (both aqueous and non-aqueous), although such a correlation is lacking for enzymes in IL systems.

In summary, based on the complexity of an enzyme's catalytic properties in ILs shown above, there is no universal method that can solve all enzyme inactivation issues. Therefore, a number of methods have been adopted or developed to improve the enzyme's stability and to increase its activity and enantioselectivity.

CONCLUSIONS

Undeniably, ILs appeared to be as promising solvents in the field of biotechnology. In particular, ILs provides a new and powerful platform for enzymes, in which they can catalyze many important reactions impossible in commonly used organic solvents. Generally the activities of biocatalyst are comparable with or higher than those observed in molecular organic solvents. In addition, enzymes also show an outstanding thermal and operational stability in ILs. Due to the complexity of an enzyme's catalytic properties in ILs, however, there is no theoretical basis that can explain enhanced performance in ILs compared to in molecular organic solvents and no universe method that can solve all enzyme inactivation issues. Nevertheless, in order to take the full advantage of the opportunities afforded by ILs in biocatalysis, there are still a number of challenges and issues needed to be addressed. One of these issue, includes several methods of stabilizing and activating enzymes in ILs that have not yet been investigated in-depth in terms of variety of enzymes and different types of ILs, therefore, more systematic studies on these subjects are necessary to explore the full potentials of ILs.

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NOMENCLATURE

Abbreviations

[$EtNH_3$] : ethylammonium
[Emim] : 1-ethyl-3-methylimidazolium
[Bmim] : 1-butyl-3-methylimidazolium
[Hmim] : 1-hexyl-3-methylimidazolium
[Omim] : 3-octyl-1-methylimidazolium
[Mmim] : 1-methyl-3-methylimidazolium
[MTOA] : methyl trioctylammonium
[Et_3MeN] : triethyl methylammonium
[PF_6] : hexafluorophosphate
[$(CF_3SO_2)_2N$]=[Tf_2N] : bis(trifluoromethylsulfonyl) amide
[BF_4] : tetrafluorobonate
[CF_3SO_3]=[TfO] : trifluoromethanesulfonate
[NO_3]: nitrate
[CH_3COO]=[OAc] : acetate
[Cl] : chlorine
[Br] : bromide
[$MeSO_4$] : methyl sulfate
[dca] : dicyanamide
[SbF_6] : hexafluoroantimonate
[MTBE] : methyl *tert*-butyl ether

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