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Mor Goldfeder & Ayelet Fishman

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MINI-REVIEW

Modulating enzyme activity using ionic liquids or surfactants

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Abstract One of the important strategies for modulating enzyme activity is the use of additives to affect their microenvironment and subsequently make them suitable for use in different industrial processes. Ionic liquids (ILs) have been investigated extensively in recent years as such additives. They are a class of solvents with peculiar properties and a "green" reputation in comparison to classical organic solvents. ILs as co-solvents in aqueous systems have an effect on substrate solubility, enzyme structure and on enzyme–water interactions. These effects can lead to higher reaction yields, improved selectivity, and changes in substrate specificity, and thus there is great potential for IL incorporation in biocatalysis. The use of surfactants, which are usually denaturating agents, as additives in enzymatic reactions is less reviewed in recent years. However, interesting modulations in enzyme activity in their presence have been reported. In the case of surfactants there is a more pronounced effect on the enzyme structure, as can be observed in a number of crystal structures obtained in their presence. For each additive and enzymatic process, a specific optimization process is needed and there is no one-fits-all solution. Combining ILs and surfactants in either mixed micelles or water-in-IL microemulsions for use in enzymatic reaction systems is a promising direction which may further expand the range of enzyme applications in industrial processes. While many reviews exist on the use of ILs in biocatalysis, the present review centers on systems in which ILs or surfactants were able to modulate and improve the natural activity of enzymes in aqueous systems.

Key words Enzyme activation · Ionic liquids · Surfactants · Mixed micelles

M. Goldfeder · A. Fishman (✉)
Department of Biotechnology and Food Engineering, Technion — Israel Institute of Technology, Haifa 32000, Israel
e-mail: afishman@tx.technion.ac.il

Introduction

The vast potential in using enzymes for industrial processes has raised the need to fine-tune or modulate their activity according to the required process. While the main strategy used to achieve this in current research is protein engineering (Bommarius et al. 2011; Kazlauskas and Bornscheuer 2009), the modification of the solvent in the immediate vicinity of the enzyme, e.g., medium engineering, proposes other wide range of possibilities (Domingez de Maria 2011).

Ionic liquids (ILs), salts that are liquids in a wide range of temperatures, are relatively novel solvents with great interest and potential for use in biocatalysis, as they have unique and tuneable properties (Gorke et al. 2010; Lai et al. 2011; van Rantwijk and Sheldon 2007). ILs have negligible vapor pressure, are thermally stable, and their chemical and physical properties can be altered by applying different anion and cation combinations. This has placed them as leading candidates in replacing conventional organic solvents.

ILs have been studied extensively in the past few years for a wide range of applications in biocatalysis. Several reviews cover the use of ILs in biphasic systems, in which they act either as the reaction media or as the extraction phase (Jutz et al. 2010; van Rantwijk and Sheldon 2007). They can also be used to coat or immobilize enzymes for improved enantioselectivity and stability (Lee and Kim 2002; Mutschler et al. 2009; Yoshiyama et al. 2013). Another aspect in which they can contribute to enzyme or protein research is their ability to improve protein crystallization and diffraction as additives or precipitating agents (Judge et al. 2009). However, the major research focus, is placed on their performance as either non-aqueous solvents or co-solvents with water. Numerous reviews describe the effect of ILs on activity and stability of enzymes (Gorke et al. 2010; Moniruzzaman et al. 2010; Naushad et al. 2012; van Rantwijk and Sheldon 2007; Yang 2009), some aiming to establish rules of thumb for the

type of effect each IL has on every enzyme. This current review will center on cases in which ILs were able to modulate and improve the natural activity of enzymes in systems in which they act as co-solvents with water. Furthermore, the means by which ILs cause these changes will be discussed.

Another type of additive which may be used as a solvent modifier is surfactants. Surfactants are amphiphilic molecules, containing a polar moiety and a hydrophobic alkyl chain. They are soluble in water, but at high enough concentrations the molecules associate to form micelles. The concentration in which this occurs is the critical micelle concentration (CMC), which is sensitive to changes in the solvent system (Otzen 2011). For example, increase in ionic strength usually results in a lower CMC, while in the presence of proteins it may be higher. In a non-polar solvent, surfactants form reverse micelles and the aqueous core of these aggregates is known as the water pool (Biasutti et al. 2008). Ionic surfactants, having a charged head group, usually denature proteins even in concentrations below their CMC due to their ability to bind and interact with both native and denatured proteins (Otzen 2011). However, there are cases in which these interactions cause enzyme activation rather than denaturation. In addition, in vivo, many enzymes are active near membranes or other protein complexes and therefore the examination of their activity in non-bound and solely aqueous environment is often not sufficient (Martinek et al. 1986).

This review will discuss the special cases in which surfactants facilitate enzyme activation and will highlight the possibility of using surfactants as additives in biocatalytic systems. There are much fewer reports in the literature on enzyme-activating surfactants than ILs in biocatalysis, since these substances are less novel and attractive. Nevertheless, it is interesting to see how surfactants too can modulate enzyme activity and possibly to find some connection between the complex effects of both ILs and surfactants.

Using ILs to improve enzyme performance in aqueous systems

The requirement for a wider range of enzyme activities and specificities encouraged studies on the ability of ILs to be used as co-solvents. The diversity in properties such as polarity, viscosity, miscibility in water or other solvents, kosmotropicity and/or chaotropicity, has made them fit for the ample diversity in enzymatic reactions which require modulation. It should be noted that the term ILs refers to a very large number and variety of solvents, and therefore one should expect diversity in the observed effect on enzymes. Miscible ILs, used as co-solvents in aqueous biocatalytic reactions, may often retain different properties than in their neat form and therefore the discussion on these aqueous systems should be differentiated than that of immiscible ILs or of non-aqueous systems. A summary of cases showing the use of ILs as co-solvents for improving enzyme performance is presented in Table 1. The examples brought here are just a few of the vast and extensive researches held on the subject. Additionally, many reports describe the inhibiting effect of ILs on enzyme activity, and these are not presented.

ILs are usually composed of a bulky asymmetric cation and a weakly coordinating anion (Domingez de Maria 2011). Figure 1 presents examples for such cations and anions; however, many more are available. The most commonly used ILs for biocatalysis have been those carrying an imidazolium-based cation, and anions including a halide (Domingez de Maria 2011; Zhao 2010). Recently, a wider range of IL types are being designed for use in enzymatic systems. For example, deep eutectic solvents (DES; Fig. 1) are considered a promising new class of solvents since they are biodegradable and more easily prepared (Domingez de Maria 2011; Domínguez de María and Maugeri 2011; Gorke et al. 2008; Lindberg et al. 2010). They are typically obtained by mixing different proportions of an ammonium salt (e.g., choline chloride) with a hydrogen donor (e.g., amines, amides, alcohols, and carboxylic acid), and share similar properties with conventional ILs. In comparison to the latter, there are currently a small number of studies on DES use in biocatalysis (Durand et al. 2013; Gorke et al. 2008; Lindberg et al. 2010; Zhao et al. 2011), but this will most likely change in the near future.

The numerous advantages of ILs over conventional organic solvents are an important driving force for their utilization in industrial processes. In comparison to organic solvents, ILs are tailorable, have negligible vapor pressure, high thermal stability, and low flammability, posing less of a hazard in their utilization (Domingez de Maria 2011; Zhao 2010). Subsequently, ILs are often considered "green" solvents, however issues of ecotoxicity and biodegradability have been addressed only in recent years (Domingez de Maria 2011). In terms of the biocatalytic system, enzymes are generally less affected by ILs compared to organic solvents (Gorke et al. 2010). Furthermore, ILs can better dissolve a wide range of substrates such as cellulose, various hydrocarbons, amino acids and more (Zhao 2010). Often, the ILs may be recycled, minimizing their effect on the environment, and reducing their cost contribution to the process.

The motivation for using ILs as co-solvents, stems from a number of reasons. The most common one is to improve the solubility of the substrates or products while the enzyme retains activity, as opposed to ordinary organic solvents in which enzymes often denature or lose activity. Improving the solubility of substrates often results in higher yields and conversion rates. For example, 1-butyl-3-methylimidazolium (BMIM) L-lactate was used as a co-solvent in an aqueous–organic solvent biphasic system and increased the solubility of hydrophobic substrates for the production of androsterone by 3α-hydrosteroid dehydrogenase from *Pseudomonas*

Table 1 List of enzymes positively affected by the presence of ILs used as co-solvents in aqueous systems

Enzyme	Origin	Reaction	Reason for IL usage	Reaction media	Effect on enzyme performance	Reference
3α-Hydroxysteroid dehydrogenase	*Pseudomonas testosterone*	Production of androsterone	Improving substrate solubility	20 % BMIM(lactate)	1.6-fold increase in activity	(Okochi et al. 2007)
Chloroperoxidase	*Caldariomyces fumago*	Enantioselective sulfoxidation of thioanisole in the presence of H_2O_2	Improving substrate solubility	50 % MMIM(Me_2PO_4)	2-fold higher conversion rate	(Chiappe et al. 2006)
Chloroperoxidase	*Caldariomyces fumago*	Enantioselective sulfoxidation of thioanisole in the presence of H_2O_2	Improving substrate solubility	20 % BMIM(Cl), BMIM(Ac), MMIM($MeSO_4$), BMPyr(Ac), BMIM(ts)	Up to 2-fold higher conversion rate	(Lichtenecker and Schmid 2009)
Laccase C	*Trametes* sp.	Mediator assisted oxidation of veratryl alcohol	Improving substrate or mediator solubility	25 % BMPyr(BF_4)	30-fold higher conversion rate	(Hinckley et al. 2002)
Laccase	*Agaricus bisporus* and *Trametes versicolor*	Oxidation of catechol	Model reaction — long-term aim to improve substrate or mediator solubility	10–20 % BMIM(Br)	Up to 2-fold increase in reaction rate (V_{max})	(Shipovskov et al. 2008)
Laccase	*Trametes versicolor*	Oxidation of 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)	Model reaction — long-term aim to improve substrate or mediator solubility	35 % MMIM($MeSO_4$) and BMIM($MeSO_4$)	1.7-fold increase in reaction rate (V_{max}) 14-fold decrease in K_M	(Tavares et al. 2012)
Lipase	*Penicillium expansum*	Hydrolysis of pNPP to pNP	Study of Hofmeister series	4.14 % w/v $NHMe_3(MeSO_4)$, NMe_4(Ac), Choline(Ac)	Up to 3.2-fold increased activity	(Lai et al. 2011)
Papain and pepsin	Hog stomach (pepsin)	Hydrolysis of casein	Florescence microscopy study	Up to 0.004 mol l^{-1} 1-(2-aminoethyl)-BIM(Br)	Up to 1.7-fold increased activity	(Fan et al. 2013)
Tyrosinase	*Bacillus megaterium*	Hydroxylation of L-tyrosine Oxidation of L-DOPA	Improved specificity	40 % $EtNH_3(NO_3)$ and BMIM(BF_4)	Up to 5-fold increase in the monophenolase/diphenolase activity ratio	(Goldfeder et al. 2013)
Trypsin, chymotrypsin, and V8-protease	Bovine	Ligation reactions of cleavage-sensitive peptide	a. Solubility of products b. Suppression of side reactions	Up to 70 % MMIM(Me_2PO_4)	Yields increase from 0–45 % to 78–87 %	(Wehofsky et al. 2008)
β-Galactosidase	*Bacillus circulans*	Transfer of galactosyl group from lactose to *N*-acetylglucosamine	Suppression of side reactions a. Improved selectivity	25 % MMIM($MeSO_4$)	2-fold increase in yield (Yield increase from 30 % to 60 %)	(Kaftzik et al. 2002)
β-Glycosylhydrolase CelB	*Pyroccocus furiosus*	Transglycosylation at 80 °C (Transfer of galactosyl group from lactose to glycerol)	a. Suppression of side reactions b. Improved selectivity	45 % MMIM($MeSO_4$)	Up to 3-fold increased selectivity Yield increase by 10 %	(Lang et al. 2006)
Lipase	*Candida antarctica*	Hydrolysis of D,L-phenylglycine methyl ester	Improved enantioselectivity	20 % BMIM(BF_4)	3-fold increase in E value	(Lou et al. 2006)
Lipase	*Candida antarctica*	Resolution of *N*-(2-ethyl-6-methylphenyl)alanine	Improved enantioselectivity	50 % ETOMG(BF_4)	*E* value increased from 16.7 to 44.7 and ee from 78.7 to 92.3	(Zheng et al. 2006)
Papain	*Carica papaya*	Asymmetric hydrolysis of D,L-*p*-hydroxyphenylglycine methyl ester	Improved enantioselectivity	12.5 % BMIM(BF_4)	1.5-fold increase in reaction rate and a 1.2-fold increase in yield of L-HPG	(Lou et al. 2005)

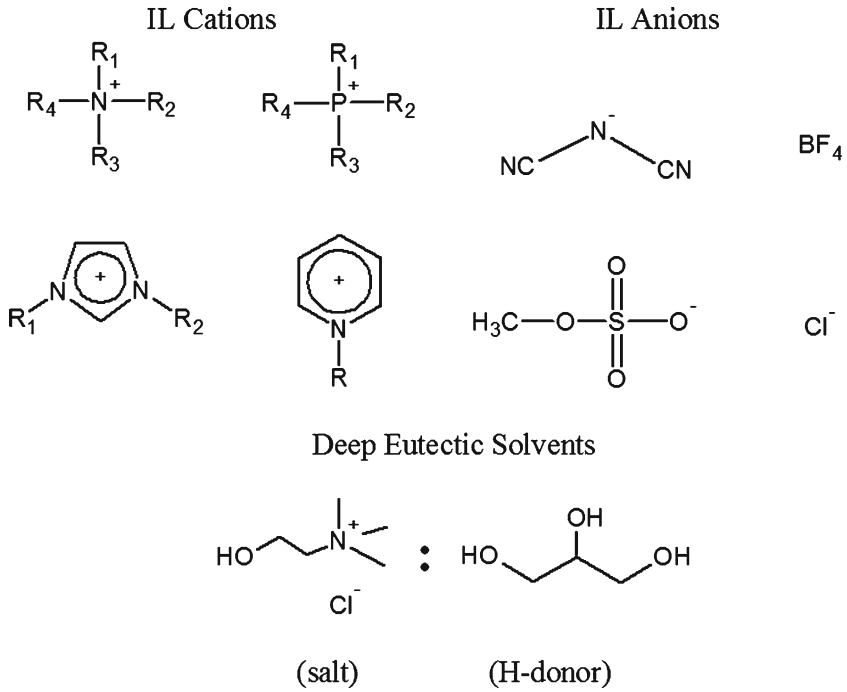

Fig. 1 Structures of typical anions and cations comprising ILs and deep eutectic solvents

testosterone (Okochi et al. 2007). The activity of the enzyme was improved 1.6-fold due to the presence of 20 % IL in the aqueous phase. In another case, the conversion rates for the sulfoxidation of thioanisole by chloroperoxidase from *Caldariomyces fumago* improved up to 2-fold in the presence of either 50 % 1,3-dimethylimidazolium dimethyl phosphate (MMIM(Me_2PO_4)) or 20 % BMIM chloride (Cl) and BMIM acetate (Ac) (Chiappe et al. 2006; Lichtenecker and Schmid 2009). An important use of ILs is in solubilizing lignin (da Costa Lopes et al. 2013; Diop et al. 2013; Magalhaes da Silva et al. 2013). Laccases have great potential for biocatalysis of water-insoluble substrates (e.g., lignin) due to their production of a free radical intermediate which further undergoes many possible reactions (Rehmann et al. 2012; Shipovskov et al. 2008). In these reaction systems improved solubility and mobility of the substrates and mediators is important, and therefore laccase activity in the presence of ILs is studied. In the mediator assisted oxidation of veratryl alcohol, laccase conversion rate improved 30-fold in the presence of 25 % 1-butyl-1-methylpyrrolidinium tetrafluoroborate (BMPyr(BF_4)) (Hinckley et al. 2002). Furthermore, laccase-catalysed oxidation of catechol was improved up to 2-fold in the presence of ILs BMIM bromide (Br) and BMIM dicyanamide ($N(CN)_2$) (Shipovskov et al. 2008). Interestingly, the improved activity was obtained at 10–20 % BMIM(Br) while in the presence of BMIM($N(CN)_2$) a milder improvement was observed only at 50–60 % IL, demonstrating the versatile effect that each IL has on the enzyme. Laccase activity was also shown to improve 1.7-fold in the presence of 35 % MMIM methyl sulfate ($MeSO_4$) and BMIM($MeSO_4$) (Tavares et al. 2012). In this case an improvement in affinity toward the substrate was also observed, as the K_M decreased by 14-fold.

Beyond the contribution to substrate solubility, there are a number of more complex effects that lead to the unexpected improvements in enzyme activities which are reported in the literature. The IL interaction with the enzyme itself should be considered, as should the effect of the ions on the water properties and on the protein–water interactions (Yang 2009). The influence of water miscible ILs on enzymes is often explained by the Hofmeister series, which is commonly used to explain the effect of salts on enzymes and is related to their kosmotropic/chaotropic properties. The suggested hypothesis is that in aqueous solutions kosmotropic anions and chaotropic cations stabilize the enzyme, while chaotropic anions and kosmotropic cations destabilize it (Yang 2009). In some cases, such as the *p*-nitrophenyl palmitate (pNPP) hydrolysis by *Penicillium expansum* lipase, the effect of ILs on activity follows the Hofmeister series well; trimethylammonium methyl sulfate ($NHMe_3$ ($MeSO_4$)) activated the enzyme 3.2-fold while BMIM($MeSO_4$), containing a more kosmotropic cation, deactivated the enzyme (Lai et al. 2011). Very recently, a fluorescence microscopy study on the interactions of the amino-functionalized IL, 1-(2-aminoethyl)-3-butylimidazolium bromide, with papain and pepsin, showed up to 1.7-fold increase in activity in the presence of IL which was attributed to the chaotropicity and hydrogen bond donating ability of the cation (Fan et al. 2013). In other cases, however, the effect may be reversed. The complexity of IL effect on enzyme activity is further observed in the case of tyrosinase. Tyrosinases perform two successive reactions and

can be active on two types of substrates, monophenols and diphenols. Yang et al. examined the effect of ILs on the diphenolase activity and found a decrease in activity for all ILs tested (Lai et al. 2011; Yang 2009). However, when the activity on both substrates was examined it was shown that in the presence of ≤40 % ethylammonium nitrate ($EtNH_3(NO_3)$) or BMIM(BF_4), the selectivity of the enzyme toward the substrates can significantly change; the commercially desired activity on the monophenol improved while the activity on the diphenol decreased (Goldfeder et al. 2013). The 5-fold increase in monophenolase/diphenolase activity ratio was shown in tyrosinase from *Bacillus megaterium* while no change or decreased activity were observed in tyrosinase from *Agaricus bisporus*. It was therefore safe to conclude that the substrate solubility could not be the main cause for the changed selectivity, and a unique effect on the enzyme's active site and structure was suggested.

Changes in enzyme selectivity are also observed in numerous studies in which ILs cause the suppression of undesired side reactions. In a reaction system containing 25 % MMIM($MeSO_4$), yields improved 2-fold for the synthesis of *N*-acetylglucosamine by β-galactosidase from *Bacillus circulans* (Kaftzik et al. 2002). In the presence of the IL, the reverse reaction, hydrolysis, was suppressed due to the reduction in water activity. Similarly, improved selectivity was shown in the presence of 45 % MMIM($MeSO_4$) for transglycosylations performed by β-glycosylhydrolase CelB from *Pyroccocus furiosus* at 80 °C (Lang et al. 2006). Another example for the suppression of a side reaction is presented in a study of protease-catalyzed ligations in the presence of up to 70 % MMIM($MePO_4$) (Wehofsky et al. 2008). The IL provided a decrease in the competing proteolytic reaction, and enabled high turnover rates compared to classical organic solvents (such as dimethylformamide) and high solubility and stability of the reactants.

The augmenting effect of ILs on enantioselectivity, especially of lipases, has been studied extensively and covered by many reviews (Domingez de Maria 2011; Gorke et al. 2010; Kim et al. 2001; van Rantwijk and Sheldon 2007). Some examples which utilized aqueous systems are described here. In the lipase-catalyzed resolution of *N*-(2-ethyl-6-methylphenyl)alanine, 50 % octymethylguanidinium (ETOMG) (BF_4) enhanced the ee (enantiomeric excess) from 79 % to 92 % (Zheng et al. 2006). The enhancement, however, came with a cost of lower conversion rates and activity. Enantioselective hydrolysis of D,L-phenylglycine methyl ester catalyzed by immobilized *Candida antarctica* lipase B (Novozyme 435) was performed in the presence of 20 % BMIM(BF_4) (Lou et al. 2006). As opposed to the previous example, at this IL concentration the enantioselectivity enhanced significantly while the activity rates did not decrease and even improved slightly. In comparison to organic solvents such as acetonitrile and 2-propanol, the activity with BMIM(BF_4) presented the best kinetic parameters (K_M and V_{max}) (Lou et al. 2006). ILs were also examined in the papain-mediated asymmetric hydrolysis of D,L-*p*-hydroxyphenylglycine methyl ester (HPG methyl ester) (Lou et al. 2005). Papain from *Carica papaya* successfully enabled both a high yield of L-HPG (47.2 %) and a high ee (95.5 %) in the presence of 12.5 % BMIM(BF_4) in comparison to aqueous or aqueous–organic solvent systems (acetonitrile, ethanol, *t*-butanol, DMSO and others). Explanations for the improved enantioselectivity are seldom dealt with in the literature. Few suggestions include the slightly modified flexibility and conformational changes of the enzyme caused by the ILs, the effect on water molecules, and the effect on the substrate state (Itoh et al. 2004; van Rantwijk and Sheldon 2007). Alternatively, the higher viscosity of ILs may slow down the undesired conformational changes leading to denaturation, thus maintaining the enzymes native structures (van Rantwijk and Sheldon 2007; Zhao 2010).

With the advancement in analytical and computational tools, researchers have recently focused on in-depth understanding of IL–enzyme interactions. Spectroscopic measurements concurrent with molecular dynamic simulations, showed that 1-allyl 3-methylimidazolium chloride (AMIM(Cl)) and 1-octyl-3-methylimidazolium chloride (OMIM(Cl)) reduce intermolecular hydrogen bonds and cause unfolding of the adenosine deaminase enzyme structure (Ajloo et al. 2013). A different conclusion was reached by Jaeger and Pfaendtner, who studied the effect of ILs on xylanase by systematically changing the IL type, the concentration of the IL–water solution, and the temperature of the in silico simulations (Jaeger and Pfaendtner 2013). These experiments indicated that likely factors in the loss of enzyme activity for this xylanase were the dampening of dynamic motion and kinetic trapping of cations in the binding pocket rather than the denaturation of the protein. Very recently, the elucidation of the link between enzyme surface charge ratio and stability in ILs has been investigated (Nordwald and Kaar 2013). This was performed by tuning the ratio of positive to negative surface charges in chymotrypsin and lipase and testing the biophysical interaction of BMIM(Cl) with them. Fluorescence quenching assays indicated that the extent of binding of the BMIM cation to chymotrypsin and lipase decreased 7- and 3.5-fold, respectively, as a function of increasing ratio of positive to negative surface charges. This resulted in destabilization of the enzymes. However, lowering the ratio of positive to negative surface charges caused the preferential exclusion of (Cl^-) which correlated with increased enzyme stability (Nordwald and Kaar 2013). These reports highlight again the intricate interplay between ILs and enzymes in aqueous environments.

In summary, ILs as co-solvents can modulate enzymatic activity by increasing substrate solubility, improving substrate

specificity, affinity and enantioselectivity, and therefore they can be applied as important additives in aqueous enzymatic reactions. The modulation of activity is caused by a complex combination of effects, differing for each reaction system. Consequently, a specific optimization process is needed for each biocatalytic system. However, the evident enhancement in enzyme performance by ILs, in most cases makes this effort worthwhile.

Using surfactants to modulate enzyme activity

Surfactants can interact with proteins in various ways and in a number of steps (Otzen 2011). Whether the surfactant's head group is charged or not will in most cases determine if these interactions will promote protein unfolding. Neutral surfactants do not denature proteins in most cases, whereas ionic surfactants do (Martinek et al. 1986; Otzen 2011). Ionic surfactants may interact with proteins through two major mechanisms: (1) electrostatic interactions between the head group of the surfactant and charged residues of the protein, and (2) hydrophobic interactions between the surfactant alkyl chain and hydrophobic residues in the protein (Savelli et al. 2000). These interactions are, for some enzymes, beneficial and may promote a desired modulation in activity (Otzen 2011; Savelli et al. 2000). Some of the reports on surfactant activation of enzymes go back two and a half decades ago. In 1987 Jones et al. showed that the activity of *Aspergillus niger* catalase is increased up to 180 % by binding of the anionic surfactant sodium dodecyl sulfate (SDS) (Jones et al. 1987). In more recent work, improved activity of α-chymotrypsin from bovine pancreas was obtained in the presence of cationic surfactants such as cetyltrimethylammonium bromide (CTAB) or *p*-octyloxybenzyltributylammonium bromide (pOOTBAB) (Alfani et al. 2000; Spreti et al. 2001). The effect was shown both below and above the CMC and in the case of CATB micelles, it has been shown that α-chymotrypsin bondage to micelles promotes conformational changes that lead to the high catalytic efficiency (Celej et al. 2004; Verma and Ghosh 2011). Similarly, both SDS and tetradecyl trimethylammonium bromide (TTAB), a cationic surfactant, were shown to significantly enhance the activity of *Thermomyces lanuginosus* lipase toward *p*-nitrophenyl butyrate (Mogensen et al. 2005). In this case as well, both tested surfactants caused the enhancement in monomer and in micelle form (at up to ~4 mM surfactant), and at higher concentrations the activity no longer increased but remained well above that obtained in buffer alone. Most lipases are activated at the water–lipid interface, and therefore the interaction of amphiphilic molecules with them is expected to affect their activity. The authors noted that the robustness of *Thermomyces lanuginosus* lipase towards ionic surfactants is not atypical since this protein is designed for amphiphilic environments (Mogensen et al. 2005). Immobilized lipases from *Pseudomonas flourescens* and from *Candida antarctica* B were examined in the presence of different surfactants and for activity on a number of substrates. When lipases are immobilized and cannot interact with hydrophobic external interfaces, the use of surfactants can preserve the open lid form of the enzyme that is important for activity. Under CMC levels, surfactants Triton X-100 and hexadecyltrimethylammonium bromide (CTAB), improved both activity (by 5- or 10-fold) and enantioselectivity (the *E* ratio increased from 40 to more than 100) (Fernandez-Lorente et al. 2007). Structural data of numerous lipases has also shown that the open lid form is stabilized by the presence of surfactants, as most of these structures were obtained from crystals grown in the presence of surfactants (Delorme et al. 2011). For example, the structure of porcine pancreatic lipase in complex with colipase was obtained in the open lid form due to the presence of tetraethylene glycol monooctyl ether (TGME) which is observed at the entrance to the active site (Fig. 2a) (Hermoso et al. 1996). Furthermore, structures of *Thermomyces lanuginosa* lipase were obtained both in the closed and open lid form depending on the time of crystal growth in the presence of C_8E_5 (polyoxyethylene ether) (Fig. 2b) (Brzozowski et al. 2000).

Nonetheless, there is an important example of an enzyme which is not designed for an amphiphilic environment and is activated by SDS–tyrosinase (also known as phenoloxidase). The reports on the enhanced activity of tyroinases in the presence of SDS are available for quite some time (Espín and Wichers 1999; Gandia-Herrero et al. 2005; López-serrano et al. 2002; Saeidian et al. 2007). The aim in most of these studies was to activate the latent form of tyrosinase present in some species. For example, a study on the effect of different surfactants on the activity of tyrosinase from dormant saffron corms (*Crocus sativus* L.) showed a significant improvement towards both monophenol (*p*-cresol, 4-fold increase) and diphenol (catechol, 2-fold increase) in the presence of up to 0.16 mM SDS (Saeidian et al. 2007). Likewise, the activity of tyrosinase from the bacterium *Marinomonas mediterranea* improved more than 2-fold on the substrate tyrosine, in the presence of 0.7 mM SDS (López-serrano et al. 2002). In both cases, and in many similar reports, the effect is contributed to conformational changes caused by the binding of SDS molecules in monomer form to the enzyme (Gandia-Herrero et al. 2005). The changes apparently involve improvement of the active site accessibility to substrates. In most studied tyrosinases, the activation does not continue above the CMC which is in the range of 0.8–1.1 mM depending on system parameters. Recently, the ability to change the selectivity of tyrosinase from *Bacillus megaterium* towards substrates in the presence of SDS was reported (Goldfeder et al. 2013). Phenol and catechol are very poor substrates for this enzyme in comparison to the less hydrophobic, tyrosine and L-DOPA.

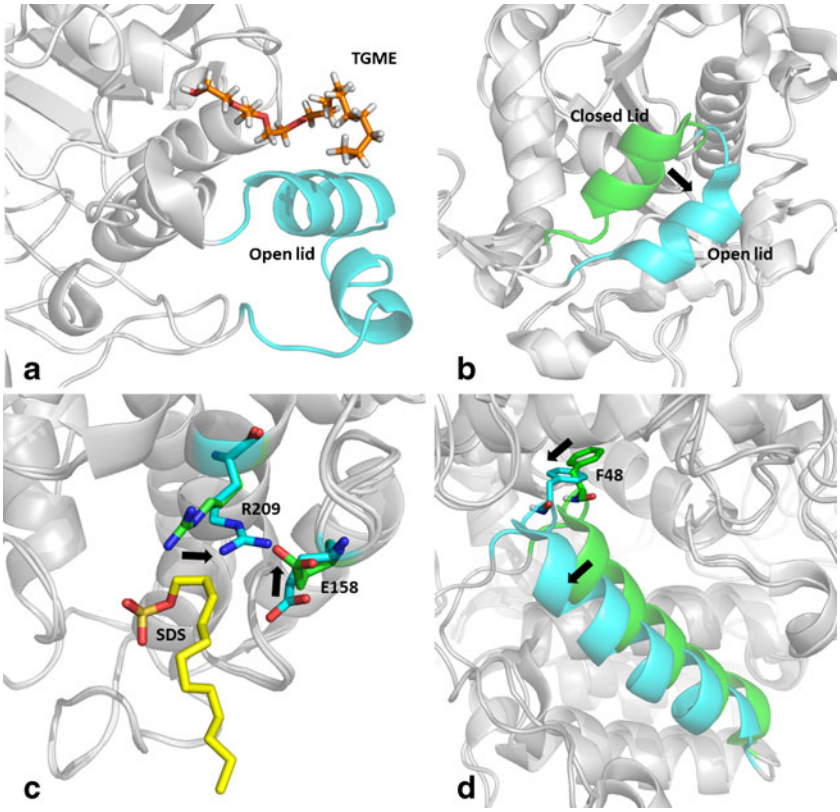

Fig. 2 Structural effect of surfactants on various enzymes. **a** Crystal structure of porcine pancreatic lipase–colipase–TMGE complex (PDB code: 1ETH). The lipase is in the open lid conformation (presented in *cyan*) due to the presence of colipase and surfactant TMGE (stick representation in *orange*). **b** Crystal structure of *Thermomyces lanuginosa* lipase in the closed (presented in *green*, PDB code: 1DTE) and open lid form (presented in *cyan*, PDB code: 1DT5) depending on the time of crystal growth in the presence of surfactant C_8E_5 (polyoxyethylene ether). The movement of the lid is pointed out by the *black arrow*. **c** Crystal structure of tyrosinase from *Bacillus megaterium* (stick representation in *green*, PDB code: 3NM8) is superimposed with a structure in complex with a molecule of SDS (stick representation in *cyan*, SDS molecule in *yellow*, PDB code: 4D87) reveals a movement in residues R209 and E158, as pointed out by the *black arrows*. **d** Scorpion hemocyanin pseudo-atomic model built based on cryo-EM density map with (stick representation in *green*, PDB code: 3IXV) and without (stick representation in *cyan*, PDB code: 3IXW) activation by SDS. A movement in the domain covering the active sight is observed, specifically in residue Phe48 with acts as a place-holder, as pointed out by the *black arrows*

In the presence of up to 50 mM SDS, a concentration well above the CMC, the activity of tyrosinase towards phenol and catechol improved 15-fold. A crystal structure determined in complex with one SDS molecule showed the movement of two residues, one of them is located in the entrance to the active site (Fig. 2c; Goldfeder et al. 2013). The movement apparently enabled the accessibility of the hydrophobic substrates. Higher SDS concentrations distorted the crystals and therefore the effect of micelles could not be examined using X-ray crystallography.

A similar enzyme in which a significant modulation in activity occurred with the addition of SDS is hemocyanin. Hemocyanins are oxygen carriers in arthropods and mollusks belonging to the same protein family as tyrosinase since they contain two copper ions in the active site. However, they have no enzymatic activity and can only bind oxygen reversibly because their active site is covered by domain with a place-holder residue that blocks the entrance of substrates (Decker and Rimke 1998). Incubation in 2 mM SDS has enabled scorpion hemocyanin to be active on both monophenols and diphenols (Nillius et al. 2008), and the transition was suggested to be induced by the presence of SDS micelles (Baird et al. 2007). Structural characterization using Cryo-EM revealed the movement of the covering domain which exposes the entrance to the active site (Fig. 2d) (Cong et al. 2009). Their data has also presented enhanced interactions on the tertiary level as well as quaternary structural changes, and therefore SDS was suggested to act as an allosteric effector for hemocyanins and other members of the type 3 copper protein family.

The similarities and joint applications of ILs and surfactants in enzymatic reactions

In aqueous systems, ILs that have cations with a long alkyl chain may behave like surfactants, for example they may form self-assembled structures (Greaves and Drummond 2008;

Heintz et al. 2010; Jungnickel et al. 2008; Qiu and Texter 2008). Furthermore, it has been shown that ILs can affect the aggregative behavior of classical surfactants. Combining the two into mixed surfactant/IL micelles provides promising new possibilities for biocatalytic applications, since the mixed micelles are much more versatile than single surfactant systems (Pinto et al. 2012). In a recent report, the evaluation of SDS/IL micelles as reaction media for the industrial synthesis of glyco-oligossacharides was described (Pinto et al. 2012). The enzyme β-galactosidase is used in industrial synthesis of glycol-oligosaccharides and is therefore important for the food industry. The use of aqueous systems resulted in low yields due to undesirable hydrolysis, and therefore other media was sought. Surfactants increased the initial reaction rate (Shin and Yang 1994) and in the presence of ILs activity similar to that in organic solvents such as ethanol or acetonitrile was obtained (Husum et al. 2001) and selectivity was enhanced (Kaftzik et al. 2002). Based on these findings, the activity of β-galactosidase from *Aspergillus oryzae* was studied in mixed micelles of SDS and BMIM(BF_4) and 1-hexyl-3-methylimidazolium (HMIM) (Cl) (Pinto et al. 2012). The properties of the formed micelles, at different SDS and IL concentrations, were examined as well. Activity was enhanced by 15–55 % in the presence of SDS/BMIM(BF_4) micelles accompanied by a decrease in K_m. The increased activity was attributed to a stabilizing effect of ILs due to their hydrogen bond capacity and to the increase in micelle size which causes positive changes in the enzyme and in its microenvironment (Pinto et al. 2012).

The combination of surfactants and ILs may also be applied through reverse micelle systems. Reverse micelles are formed by surfactants in non-aqueous media such as organic solvents. For use in biocatalysis, enzyme molecules are encapsulated in the aqueous phase called the water pool, formed within the micelles (Biasutti et al. 2008; Carvalho and Cabral 2000), and this prevents the loss of activity often caused by the organic solvent media. The activity of enzymes in reverse micelles of surfactants has been studied for the past few decades (Chen et al. 2001; Das et al. 2008), but the addition of ILs to these systems, forming water-in-IL microemulsions, is quite recent (Mehta and Kaur 2010; Moniruzzaman et al. 2008b; Pavlidis et al. 2009). Enzyme activity in water immiscible ILs can be highly improved by the formation of these microemulsions and this is made possible by the presence of surfactants. Tween 20 and Triton X-100 were used to form water-in-IL microemulsions in BMIM hexaflourophosphate (PF_6) and the lipase catalyzed esterification reaction was examined in the resulting systems (Pavlidis et al. 2009). Lipases from *Candida rugosa*, *Candida viscosum* and *Thermomyces lanuginosa* exhibited higher catalytic activity and stability in these systems compared to other microheterogeneous media such as AOT (sodium bis-(2-ethylhexyl) sulfosuccinate) microemulsions in organic solvent. In another study, AOT was used to form water-in-IL microemulsions in BMIM(PF_6), and the hydrolysis of 4-nitrophenyl butyrate by lipase from *C. rugosa* was examined (Xue et al. 2012). The catalytic efficiency was 14.3-fold higher than in the water saturated IL system, due to the significant decrease of K_M in the microemulsion. Goto et al. used a microemulsion system of AOT/OMIM bis(trifluoromethylsulfonyl)amide)(Tf_2N)/water/1-hexanol for the oxidation of pyrogallol by horseradish peroxidase, and reported that the reaction was is up to 5-fold more effective than in a conventional AOT/water/isooctane microemulsion (Moniruzzaman et al. 2008a). These studies indicate that the water-in-IL microemulsion systems are very promising for biocatalytic reactions in non-aqueous media. They provide a large interface and a high water activity which are necessary for the enzymes, especially in the case of lipases, and the IL can often provide an activating effect of its own (Xue et al. 2012). Furthermore, the enzyme structure may be affected by the water-in-IL microemulsion system as shown for lipase by FTIR and circular dichroism spectroscopy, causing it to be more rigid in comparison to the enzyme structure in other microheterogeneous systems (Pavlidis et al. 2009).

Conclusions and future perspectives

This review has highlighted the increasing possibilities and potential in modulating enzyme activity and selectivity with the use of surfactants or ILs as co-solvents. There are various mechanisms by which ILs in aqueous reaction systems can manipulate enzymatic performance, and recent studies have shown that they are a complex combination of effects depending on the specific system. Future advancement in basic research dealing with IL–enzyme interactions may expedite rational design of beneficial ILs and promote their application in biocatalysis. Additional success stories will also be crucial to the progress towards actual industrial realization. The use of surfactants to modulate enzyme activity is not straightforward as well. Usually known as denaturing substances, surfactants can promote positive changes in some enzymes. These modulations caused mainly by conformational changes can lead to important new applications. The incorporation of both ILs and surfactants in reaction systems, as either mixed micelles or as water-in-IL microemulsions has been shown to be very promising, and will most likely become more prevalent in future studies.

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