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Review

Effect of ions and other compatible solutes on enzyme activity, and its implication for biocatalysis using ionic liquids

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Abstract

The effect of ions on enzyme activity and stability usually follows the Hofmeister series (or the kosmotropicity order): kosmotropic anions and chaotropic cations stabilize enzymes while chaotropic anions and kosmotropic cations destabilize them. The effect of ionic liquids (ILs) on the enzyme activity/stability/enantioselectivity is complicated especially when there is no or little water presence in the IL media. However, when aqueous solutions of hydrophilic ILs are employed as reaction media, the enzyme seems to follow the Hofmeister series since ILs dissociate into individual ions in water.

Keywords: Enzyme; Activity; Stability; Ion; Hofmeister series; Ionic liquid

Contents

1. Introduction

More than a century ago, Hofmeister [1,2] noticed that ions exhibited various ability of precipitating the protein. The sequence of the ion ability in stabilizing proteins is well known as the Hofmeister series (Fig. 1) although later this concept was also introduced into other areas such as physical, colloid, polymer and surface chemistry [3,4].

The effect of ions on protein stability may be caused by 'chemical' interactions (or chelation) between proteins and ions to form complexes, such as ions being used as substrate, co-substrate or co-factors of enzymes [9–13]. However, the ion specificity was mostly attributed to the ion ability to modify the water structure ('physical' effect), thus influences the protein hydration environment [6,9,14–23]. The strongly hydrated ions that increase the structure of water are called kosmotropes ('structure-makers'), and those weakly hydrated ions that decrease the structure of water are known as chaotropes ('structure-breaker'). Kosmotropes are usually small and highly charged, while chaotropes are large and low-charged. In fact, all multivalent ions are highly hydrated, therefore, are kosmotropic [24]. Empirically, singly charged chaotropic ions are usually those ions with radii larger than 1.06 Å for cations and larger than 1.78 Å for anions [16]. However, there are many exceptions to this simple rule, especially those organic ions [25].

Water may be described as a unique hydrogen-bonded polymeric structure of low entropy with two types of local structures: low density water (LDW) and high density water (HDW)

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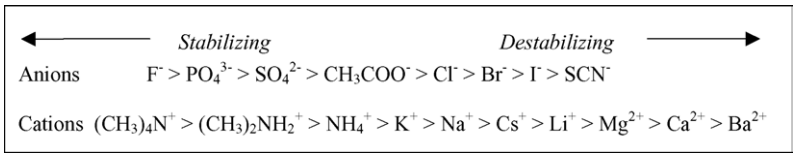

Fig. 1. The Hofmeister series as an order of the ion effect on protein stability [5–8].

[26–30]. There is an equilibrium between LDW and HDW as illustrated in the following:

Low density water (LDW) ⇌ High density water (HDW)

Kosmotropes tend to accumulate in HDW and shift the equilibrium to the left, while chaotropes selectively partition into LDW and shift the equilibrium to the right [30,31]. Through years of studies on proteins and other biological molecules [5,14,16,31], it has been realized that strong kosmotropic anions stabilize proteins and strong kosmotropic cations destabilize them [5,17,21,22,32,33]. Therefore, an optimal stabilization of biological macromolecules (including enzymes) could be achieved through the use of salts with kosmotropic anions and chaotropic cations [5,17,22], i.e., different biological molecules may require different LDW/HDW equilibriums; the optimal stabilization could be achieved through a selection of salts with kosmotropic anions and chaotropic cations [5,17,22]. For different biological macromolecules, however, ions do not necessarily stabilize or destabilize them in the same kosmotropicity order [5,29,34].

The kosmotropicity of ions can be quantified by the viscosity *B*-coefficients and other parameters (such as hydration entropies, hydration volumes, heat capacities, NMR B'-coefficients and ion mobility, etc.) [25]. Kosmotropes usually have positive *B*-coefficients since strongly hydrated ions exhibit a larger change in viscosity with concentration, while chaotropes have negative *B*-coefficients due to the weak hydration [35]. The *B*-coefficients are usually available for many inorganic ions [35], therefore, the order of ion kosmotropicity based on *B*-coefficients was shown in Fig. 2. In conclusion, there is a general agreement between this kosmotropicity sequence and the Hofmeister series in Fig. 1 with a few exceptions (as described in the caption of Fig. 2).

Although numerous reviews on the Hofmeister series of proteins were reported as indicated above, there are limited summaries of the ion effect on the enzyme activity, including one review on the enzymatic catalysis and colloidal structures [9], and another review on the anion selectivity in biological systems [15]. Meanwhile, with the fast growing research of biocatalysis using ionic liquids (ILs) [36–48], there is an urgent need of understanding the mechanism of enzymatic reactions in these novel 'green' media. This paper is intended to provide such a connection between the Hofmeister series and the enzymatic activity in ILs.

2. Ion effect on the enzyme activity

Table 1 summarized some typical examples of the ion effect on the enzyme activity and stability. These ions may cause competitive or non-competitive inhibition of enzymes [52,56]. Closely following the Hofmeister series, ions may impose inhibition or activation on enzymes as illustrated in Table 1. Halophilic enzymes, such as menadione reductase from *Halobacterium cutirubrum*, require high concentrations of salts to maintain their high activity and stability. In the case of menadione reductase, the enzyme exhibited the optimal activity in 2–3 M NaCl solutions [57].

Many earlier studies of the ion specificity focused on the activity of carbonic anhydrase, an enzyme discovered in the red blood corpuscles mammals by Meldrum and Roughton [58]. Most of these results were in consistent with the Hofmeister series and the order of ion kosmotropicity with a few exceptions. Most studies (Table 1) also concluded that kosmotropic anions and chaotropic cations stabilize enzymes, while chaotropic anions and kosmotropic cations destabilize them. Many other experiments also supported this conclusion. For example, kosmotrope CF_3COONa used as additives was able to refold the unfold 434 repressor in 7 M urea [59]. $[CF_3COO]^-$ ions seem to 'drag' water molecules away from the protein through ordering, which allows the protein to refold. Another study revealed that the degree of activation of *Subtilisin* Carlsberg during the lyophilization is 2800-fold when using the solution of kos-

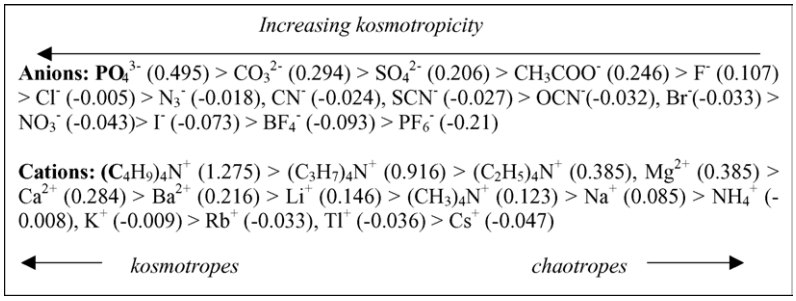

Fig. 2. The order of kosmotropicity solely based on *B*-coefficients [35] of ions (shown in parentheses next to each ion). $(CH_3)_4N^+$ is a chaotrope despite its large *B*-coefficient (due to the hydrophobic hydration [22,33,49–51]). CN^- and SCN^- are strong enzyme inhibitors and thus should be stronger chaotropes than Br^-, NO_3^- and I^- [21,52–55].

Table 1
Effect of ions on the activity and stability of enzymes

Nature of effect	Order of effectiveness ←Stabilization/Activation Destabilization/Denaturation→	Ref.
Effect of neutral salts on the activity of carbonic anhydrase in 0.024 M phosphate, pH 7.5	*Anions*: [cacodylate]$^-$ > CH_3COO^- > SO_4^{2-} > NO_3^- > Cl^- > Br^- > I^- (Na^+ or K^+ salts)	[76]
Inhibition of enzyme D amino acid oxidase by salts	*Anions*: F^- > Cl^- > NO_3^- > Br^- > I^-	[77]
Inhibition of bovine carbonic anhydrase by salts	*Anions*: F^- > Cl^- > NO_3^- (K^+ or Na^+ salts)	[78]
Anions inhibition of xanthine oxidase by urea	*Anions*: SO_4^{2-} > F^- > CH_3COO^- > Cl^- > Br^- > SCN^- (K^+ salts)	[15,52]
Inhibition of acetoacetic decarboxylase by anions	*Anions*: CCl_3COO^- > F^-, IO_3^- > BrO_3^- > Cl^- > Br^- > ClO_3^- > NO_3^- > I^- > ClO_4^- > SCN^- > HSO_3^-	[53]
Thermal stabilization of bovine pancreatic ribonuclease by salts (0.5–3.0 M)	*Cations*: $(CH_3)_4N^+$ > $(C_2H_5)_4N^+$ > $(C_3H_7)_4N^+$ > $(C_4H_9)_4^+$ (Br^- salts)	[21]
Inhibiting the activity of myosin nucleoside triphosphatase, trypsin, lactate dehydrogenase, estradiol-17β dehydrogenase and fumarase by salts (0.3–3.0 M)	*Anions*: CH_3COO^- > Cl^- > NO_3^- > Br^- > I^- > SCN^- > ClO_4^- (K^+ or Na^+ salts) *Cations*: $(CH_3)_4N^+$ > Cs^+ > K^+ > Na^+ > Li^+ (Cl^- salts)	[79]
Inhibiting the activity of trypsin, α-chymotrypsin, renal acylase, wheat germ lipase, estradiol-17β dehydrogenase, β-amylase and β-galactosidase using uncharged substrates by salts (0.5–2.0 M)	*Anions*: CH_3COO^- > Cl^- > Br^-, NO_3^- > I^- > ClO_4^-, SCN^- (K^+ or Na^+ salts)	[80]
Inhibition of esterase action of carbonic anhydrase B	*Anions*: SO_4^{2-} > Cl^- > I^- > SCN^-	[81]
Inhibition of esterase activity of bovine carbonic anhydrase for the hydrolysis of *p*-nitrophenylacetate	*Anions*: F^- > Cl^- > CH_3COO^- > Br^- > NO_3^- > HCO_3^-, HSO_3^- > I^-, ClO_4^- > CNO^-, SCN^-, N_3^- > HS^- > CN^-	[54,82,83]
Inhibition of Sipunculus and Solen arginine kinases	*Anions*: CH_3COO^- > Cl^- > Br^- > SCN^-, CCl_3COO^- > ClO_4^- > NO_3^- (K^+ salts)	[56]
Promoting the activity of menadione reductase by salts	*Anions*: Cl^-, $H_2PO_4^-$ > Br^- > NO_3^- > ClO_4^-, SCN^- (Na^+ or K^+ salts)	[57]
Anion inhibition of firefly luciferase	*Anions*: Cl^- > Br^- > I^-, NO_3^- > SCN^- (Na^+, K^+ or NH_4^+ salts)	[84]
Activation of succinate dehydrogenase by anions	*Anions*: Membrane-bound: I^- > $HCOO^-$ > Br^- > Cl^-, NO_3^-, ClO_4^- > HPO_4^-, CH_3COO^- (Na^+ or K^+ salts) Soluble: SO_4^{2-} > Cl^- > CH_3COO^- (Na^+ salts)	[85]
Effect of anions on hydration and dehydration of CO_2 catalyzed by human red cell carbonic anhydrases B and C	*Anions*: Hydration: F^- > Cl^- > Br^- > HCO_3^- > NO_3^- > I^- > ClO_4^- > SCN^- > CNO^- (carbonic anhydrase B); F^- > HCO_3^- > NO_3^- > Cl^- > Br^- > ClO_4^- > I^- > SCN^- > CNO^- (carbonic anhydrase C) Dehydration: F^- > Cl^- > NO_3^- > Br^- > ClO_4^- > I^- > SCN^- > OCN^- (carbonic anhydrase B); Cl^-, Br^- > F^- > I^- > NO_3^- > ClO_4^- > SCN^- > OCN^- (carbonic anhydrase C)	[15,86]
Cation- and anion-dependent reassociation of formyltetrahydrofolate synthetase subunits	*Anions*: SO_4^{2-} > Cl^- > CH_3COO^- > NO_3^- > Br^- > I^- > ClO_4^-, SCN^-, CCl_3COO^- (NH_4^+ salts) *Cations*: NH_4^+ > Tl^+ > Rb^+, K^+ > Cs^+ > Na^+, Li^+ (Cl^- salts)	[87]
Effect of salts on the maximal velocity and activation volume of the M_4-lactate dehydrogenase reaction	*Anions*: F^- > SO_4^{2-} > Cl^- > Br^- > I^- > SCN^- (K^+ or Na^+ salts) *Cations*: K^+ > Na^+ > Li^+ (Cl^- salts)	[88]
Activation of pig liver phosphofructokinase	*Anions*: $S_2O_3^{2-}$ > SO_4^{2-} > SO_3^{2-} > MnO_4^{2-} > NO_3^- > Cl^- > ClO_3^-, Br^- > I^- > SCN^- (Na^+ salts)	[65]
Effect of salt on the Michaelis-Menten constant of HIV-1 protease	*Anions*: SO_4^{2-} > CH_3COO^- > Cl^- > Br^- > I^- *Cations*: NH_4^+ > K^+ > Na^+	[89]
Activation of Herpes Simplex Virus type 1 (HSV-1) protease by antichaeotropic salts (0.5–2.5 M)	*Anions*: PO_4^{3-} > SO_4^{2-} > CH_3COO^- ≫ Cl^- (Na^+, K^+, NH_4^+ or Mg^{2+})	[90]
Thermal stability and quaternary conformation of pig heart mitochondrial malate dehydrogenase by salts (0.05–2.0 M)	*Anions*: citrate^{3-} > SO_4^{2-}, tartrate^{2-} > PO_4^{3-} > F^-, CH_3COO^- > Cl^- > Br^- (Cs^+, K^+, Na^+, NH_4^+ and $(CH_3)_4N^+$ salts) *Amino acids*: NaGlutamate, NaAspartate > NaGlycinate > lysine·HCl > arginine·HCl	[91]

Table 1 (*Continued*)

Nature of effect	Order of effectiveness ←Stabilization/Activation Destabilization/Denaturation→	Ref.
Activation of Herpes Simplex Virus type 1 (HSV-1) protease by kosmotropes (0.2–0.8 M)	*Anions*: citrate^{3-} > [isocitrate]$^{3-}$ > $HP_2O_7^{3-}$ > $PO_4^{2-/3-}$ > [*cis*-aconitate]$^{3-}$ > SO_4^{2-} > [D-malate]$^{2-}$, [L-malate]$^{2-}$ > [succinate]$^{2-}$ > [D-glutamate]$^{2-}$ > [L-glutamate]$^{2-}$ > CH_3COO^- > Cl^- > Br^-, > I^- > ClO_4^- (Na^+ salts)	[62,92]
Thermoinactivation of glucose dehydrogenase	*Cations*: K^+ > Na^+ > NH_4^+ > Li^+ (Cl^- and Br^- salts)	[93]
Salt-induced activation of lyophilized subtilisin Carlsberg in hexane	*Anions*: CH_3COO^- > SO_4^{2-} > F^- > Cl^- > Br^- > I^- (Na^+ salts)	[60]
The effectiveness of monovalent cations in stabilizing glucose oxidase against urea and thermal denaturation	*Cations*: K^+ > Na^+ > Li^+ (Cl^- salts)	[94]
Dependence of activity of NADH oxidase from *Thermus thermopilus* on temperature and urea in the presence of salts (0–3.0 M)	*Anions*: (on temperature) $H_2PO_4^-$ > SO_4^{2-} > I^- > Cl^- > CH_3COO^-, Br^- > ClO_4^- > SCN^- (Na^+ or K^+ salts) (on urea) SO_4^{2-} > $H_2PO_4^-$ > Cl^- > ClO_4^- > I^- > SCN^- (Na^+ or K^+ salts) (catalytic activity in 1 M salt solution) Cl^- > Br^- > I^- > CH_3COO^- > SO_4^{2-}, $H_2PO_4^-$ > ClO_4^-, SCN^- (Na^+ or K^+ salts)	[55]
Salt activation of prostate specific antigen (PSA)	*Anions*: SO_4^{2-}, citrate^{3-} > CH_3COO^- > Cl^- > Br^- > I^- (Na^+ salts) *Cations*: Na^+ > Li^+ > NH_4^+ > K^+ > Mg^{2+} (SO_4^{2-} salts)	[95]
The hydrolytic activity of *Aspergillus niger* lipase	*Anions*: Br^- > Cl^-, NO_3^- > ClO_4^- (Na^+ salts)	[4]
Effect of salts on activity of *Candida rugosa* lipase	*Cations*: Li^+ > Na^+ > K^+ (Cl^- salts, water activity 0.33 and 0.84)	[96]
Effect of salts on catalytic activity of alkaline phytase from lily pollen	*Anions*: Cl^- > SO_4^{2-} (Na^+, K^+, NH_4^+ and Mg^{2+} salts) *Cations*: K^+ > NH_4^+ > Na^+ > Mg^{2+} (Cl^- salts)	[97]

motropic CH_3COONa than in the salt-free solution, and the optimal activation was achieved through using binary mixtures of different salts [60,61]. The kosmotropic anions (such as citrate or phosphate) could increase the activity of a protease over 10-fold [62]. Even some simple salts (such as NaCl, KCl and CsCl) were found to enhance the stability of dihydrofolate reductases [63] and HIV-1 protease [64]. However, the order of ions in stabilizing various enzymes may be different depending on the specific application (Table 1).

Multivalent anions are stronger kosmotropes, therefore, they are better enzyme stabilizers or activators (Table 1). For instance, a study conducted by Foe and Trujillo [65] showed that divalent anions (such as $S_2O_3^{2-}$, SO_4^{2-}, SO_3^{2-} and MnO_4^{2-}) are activators for the pig liver phosphofructokinase, while monovalent anions are not. In fact, SO_4^{2-} has been long known not only as an enzyme stabilizer [66–68], but also an activator of human platelet [69], rat erythrocyte [70], rat jejunum [71], pea seed [72] and blood fluke enzymes [73]. A study by Ramos and Baldwin [74] reported a higher thermal stability of native ribonuclease A achieved when sulfate anions (0–1 M) were used. Similarly, Fayos et al. [75] observed that sodium phosphate is capable of enhancing the thermal stability of the IGg binding domain of protein L from *Peptostreptococcus magnus* (Prot L).

Most references in Table 1 also indicated that cations have less influence on the enzyme activity than anions do. For example, sulfate, citrate and acetate could increase the activity of prostate specific antigen (PSA) several hundred folds, while cations (Li^+, Na^+, K^+, NH_4^+ and Mg^{2+}) had a modest effect [95]. However, it is not always the case: (1) when the enzyme surface is negative charged, the cations may have considerable interaction with the enzyme. Glucose oxidase from *Aspergillus niger* is an acidic dimeric enzyme that has a negative charged surface. The monovalent cations (K^+, Na^+ and Li^+) influenced the enzyme activity and tertiary structure, but not the secondary structure [94]. This monovalent cation-stabilized enzyme was less active than the native enzyme, but has a higher stability against urea and thermal denaturation following the Hofmeister series (Table 1). (2) When a cation is the co-factor to form ion-enzyme complexes, the cation may be essential for the enzymatic activity. For example, carbonic anhydrase is a Zn compound where Zn is part of the active site of the enzyme [98]. A further study also indicated that Zn(II) and Co(II) were efficient reactivators in restoring esterase activity of bovine carbonic anhydrase to the apoenzyme while other metal ions were not effective in activating this enzyme [10]. As another example, Tl(I) was reported being able to serve as the required monovalent cation in the activation of rabbit muscle pyruvate kinase [11].

However, at high concentrations, it was believed that both kosmotropic and chaotropic anions inhibit the enzyme by different mechanisms [55]. Kosmotropic anions decrease the apparent Michaelis constant and increase the activation barrier, while chaotropic ones have the opposite effect. The active site flexibility is important in the function of the enzyme. The kosmotropic anions induce high rigidity, while chaotropic anions induce

high flexibility. Both situations lowered the enzyme activity [55].

3. Effect of other compatible solutes on the enzyme activity

Besides those net-charged ions discussed previously, there are other types of ions (such as zwitterions) and solutes that may also affect the enzyme activity. These substances are so called compatible solutes. The compatible solutes were defined by Brown and Simpson [99] as low molecular weight and neutral compounds that cause little enzyme inhibition at high concentrations. In general, they do not cause disturbance of biological systems. Among compatible solutes, those who further improve the stability of biological systems are called compensatory solutes because they allow organisms to overcome extreme conditions such as high ionic strength, high or low temperature, drying and the presence of denaturants such as urea, arginine and guanidine (including guanidinium ions) [52,100–103]. Compensatory solutes usually have high solubility in water, have no net charge, and do not interact with proteins. Some compensatory solutes are also known as osmolytes. Osmolytes (or osmoregulatory solutes) refer to solutes in nature that are used to maintain the intracellular osmotic pressure. All known osmolytes are also compatible solutes by varying degrees [104,105].

Generally, there are three types of compensatory solutes (some of them are called compensatory kosmotropes) [104,106]: (1) inorganic ions (discussed previously); (2) carbohydrates (such as sugars), polyhydric alcohols or polyols (sorbitol and trehalose); (3) zwitterions containing a relatively hydrophobic cationic region including amino acids (α-, β- or γ-) [107–110], and quaternary ammonium compounds (such as glycine betaine, extoine and trimethylamine oxide known as TAMO). The reason that these compounds can be used as compatible solutes was explained as the resemblance between these compounds and those cations and anions in the Hofmeister lyotropic series (for example, glycine resembles ammonium acetate; taurine resembles ammonium sulfate) [103].

Compensatory solutes have been extensively studied in the protein stabilization [102–106,111–114]. Meanwhile, there have been some attempts of using compensatory solutes to enhance the enzyme performance. Firstly, amino acids can be enzyme stabilizers. For example, the effect of two amino acids (glycine and β-alanine) on the esterase activity of bovine carbonic anhydrase was found similar to that of kosmotropic acetate anions [82]. Salts of amino acids were investigated as effective solutes in stabilizing the pig heart mitochondrial dehydrogenase (*phm*-MDH) against temperature induced changes; the order of stabilization is NaGlutamate, NaAspartate > NaGlycinate > lysine·HCl > arginine·HCl [91]. *N*γ-Acetyldiaminobutyrate (NADA) was shown having a greater ability in protecting the rabbit muscle lactate dehydrogenase against thermal inactivation than ectoine or potassium diaminobutyrate [108]. Glycine, alanine and proline (as well as TAMO and betaine) displayed non-perturbing or favorable effects on the enzyme–substrate and enzyme–co-factor complex formation, catalytic velocity and protein structural stability [107].

Secondly, various alcohols are also stabilizers of enzymes. Polyols increased the thermo-stability of halophilic enzymes in an increasing order of glycerol < erythritol < xylitol < sorbitol. The overall hydroxyl group concentration was related to the effectiveness of polyols on the stabilization [93]. The presence (2–5%, v/v) of alcohols as co-solvents had a strong influence on the activity of Herpes Simplex Virus type 1 (HSV-1) protease. An increasing enzyme activity was observed in alcohols in the order of ethanol < methanol < trifluoroethanol and isopropyl alcohol < ethyl glycol < glycerol < sorbitol [92]. Diglycerol phosphate possessed a strong ability in stabilizing enzymes (rabbit muscle lactate dehydrogenase, baker's yeast alcohol dehydrogenase and *Thermococcus litoralis* glutamate dehydrogenase) against thermal inactivation [115].

Examples of other compensatory solutes in stabilizing the enzyme include: malate dehydrogenase was not inhibited by glycine betaine with concentrations up to 500 mM [116]; glycine betaine and disaccharides (sucrose, trehalose and maltose) exerted a remarkable effect in stabilizing lactic dehydrogenase (LDH) and phosphofructokinase (PFK) against heating, freezing and drying [117].

4. Effect of ILs on enzyme activity and the Hofmeister series

Most biocatalysis in ILs involved no or little water as co-solvent [36–47]. Most ILs used in these applications are hydrophobic types of PF_6^- and $(CF_3SO_2)_2N^-$ salts (water-immiscible or partially water-miscible). Hydrophobic solvents could be superior to hydrophilic ones (water-miscible) because the latter might remove internally bound water (essential water) from the enzyme [118]. The enzyme was practically suspended rather than dissolved in the hydrophobic media. Currently, there are limited results of enzymatic reactions in hydrophilic ILs containing a considerable amount of water [38,119–124]. Table 2 summarized the effect of cations and anions of ILs on the enzyme activity and stability. Instead of a systematic comparison of the effect of various ILs, many of current research on biocatalysis in ILs only studied some 'isolated' ILs. Therefore, Table 2 was unable to include these examples for the comparison purpose.

When the biocatalysis was conducted in hydrophobic or anhydrous hydrophilic ILs (Table 2), the enzyme activity does not seem to follow the Hofmeister series [125] even though NO_3^- (*B*-coefficient = −0.043), BF_4^- (−0.093) and PF_6^- (−0.21) are all chaotropic anions. The same conclusion applies to the enantioselectivity of enzymes [126–128]. The hydrophobicity of ILs may be described by the log *P*, a concept derived from the partition coefficient of ILs between water and octanol. Generally, enzymes are more stable in solvents with a larger log *P* (>3) (such as hexane has a log *P* of 3.9) than lower log *P* (such as ethanol has a log *P* of −0.24) [129]. Free lipase (*Candida rugosa*) was found only active in [BMIM][PF_6] (log *P* = −2.39) for the transesterification of methyl methacrylate, but inactive in other ILs

Table 2
Effect of ILs on the enzyme activity and stability

Nature of Effect	Order of effectiveness ←Stabilization/Activation Destabilization/Denaturation→	Ref.
Hydrophobic or anhydrous ILs		
Stability of Novozym 435 in ILs at 30 °C	*Anions*: CH_3COO^- > PF_6^- > NO_3^- ($[BMIM]^+$ or $[MMEP]^+$ based ILs) *Cations*: $[MMEP]^+$ > $[BMIM]^+$ (CH_3COO^-, PF_6^-, or NO_3^- salts)	[130]
Initial reaction rates of PEG-lipase catalyzed alcoholysis in ILs	*Cations*: $[OMIM]^+$ > $[HMIM]^+$ > $[BMIM]^+$ (PF_6^- based ILs)	[165]
Enantioselective acylation of 1-phenylethanol by lipase CaLB	*Anions*: $[(CF_3SO_2)_2N]^-$, $[CF_3SO_3]^-$ > BF_4^- > PF_6^- ($[BMIM]^+$ based ILs) *Cations*: $[OMIM]^+$ > $[HMIM]^+$ > $[BMIM]^+$ (BF_4^- based ILs)	[166]
Activity of PEG-lipase PS complex in ILs	*Cations*: $[OMIM]^+$ > $[HMIM]^+$, $[BMIM]^+$ (PF_6^- based ILs)	[167]
Stability of β-galactosidase at 50 °C in 100% ILs	*Cations*: $[MMIM]^+$ > $[BMIM]^+$, $[MNEt_3]^+$ ($MeSO_4^-$ based ILs)	[120]
Enantioselectivity of the acetylation of 1-phenylethanol with vinyl acetate by lipase from *Pseudomonas cepacia* in ILs (purification method B, no additive)	*Anions*: BF_4^- > PF_6^- ($[BMIM]^+$ based ILs) *Cations*: $[EMIM]^+$ > $[PrMIM]^+$, $[BuPy]^+$ > $[PrPy]^+$ > $[BMIM]^+$ (BF_4^- based ILs)	[168]
Enantioselectivity of *Candida rugosa* lipase in the esterification of 2-substituted-propanoic acids and 1-butanol in ILs	*Anions*: PF_6^- > BF_4^- ($[BMIM]^+$ based ILs) *Cations*: $[BMIM]^+$ > $[OMIM]^+$ (PF_6^- based ILs)	[131]
Activity of *Candida antarctica* lipase B in transesterification of ethyl butanoate and 1-butanol	*Anions*: BF_4^- > PF_6^- > $[lactate]^-$ > NO_3^- ($[BMIM]^+$ based ILs)	[125]
Hydrophilic ILs containing water		
Specific activity of esterase from *Bacillus stearothermophilus* in the kinetic resolution of 1-phenylethanol (10 mM) with vinyl acetate (200 mM) at 40 °C, a_w = 0.11	*Anions*: $[(CF_3SO_2)_2N]^-$ > BF_4^- > PF_6^- ($[BMIM]^+$ based ILs)	[169]
Stability of α-chymotrypsin in ILs (2% water, v/v) at 50 °C	*Anions*: PF_6^- > BF_4^- ($[BMIM]^+$ based ILs)	[123]
Stability of *Candida antarctica* lipase B in ILs (2% water, v/v) exhibited by incubation without substrates	*Anions*: BF_4^- > PF_6^- > $[(CF_3SO_2)_2N]^-$ (($[EMIM]^+$ and $[BMIM]^+$ based ILs) *Cations*: $[MMIM]^+$ > $[BMIM]^+$ ($[(CF_3SO_2)_2N]^-$ based ILs)	[124,133]
Activity of β-galactosidase and Subtilisin protease Savinase™ in 50% $[BMIM][BF_4]$ aqueous solutions	In 50% $[BMIM][BF_4]$, the activity of β-galactosidase was only 5.70% of that in borate buffer (pH 9) and the activity of Savinase™ was 37.5% of that inborate buffer (pH 9).	[38]
Activity of cellulose from Trichoderma reesei in salt solutions containing 20–100% water	Sodium citrate buffer > sodium dodecylsulfate > NaCl > [BMIM]Cl	[154]
Activity of formate dehydrogenase and β-galactosidase in 25–75% ILs	*Anions*: $MeSO_4^-$ > NO_3^-, BF_4^- ($[MMIM]^+$, $[BMIM]^+$, $[PrNH_3]^+$ salts)	[120]
Stability of 'Amano' protease P6 in 15% (v/v) ILs	*Anions*: CH_3COO^- > CF_3COO^- > OTs^- > BF_4^- ($[EMIM]^+$ or $[BuPy]^+$ based ILs) *Cations*: $[EMIM]^+$, $[BuPy]^+$ > $[BMIM]^+$ > $[EtPy]^+$ (CF_3COO^- based ILs)	[142,143]

Note: BMIM, 1-ethyl-3-methylimidazolium; MMEP, 1-methyl-1-(2-methoxyethyl)pyrrolidinium; OMIM, 1-*n*-octyl-3-methylimidazolium; HMIM, 1-*n*-hexyl-3-methylimidazolium; MMIM, 1,3-dimethylimidazolium; EMIM, 1-ethyl-3-methylimidazolium; PrMIM, 1-propyl-3-methylimidazolium; BuPy, 1-*n*-butylpyridinium; PrPy, 1-propylpyridinium; EtPy, 1-ethylpyridinium.

including $[BMIM][CH_3COO]$ ($\log P = -2.77$) $[BMIM][NO_3]$, ($\log P = -2.90$) and $[BMIM][CF_3COO]$ [130]. This example illustrated that the high hydrophobicity (large $\log P$) of ILs could be beneficial to the enzyme activity. It was possible that the enzyme was destabilized by the latter three ILs because they are hydrophilic and could strip off the essential water from the enzyme at high salt concentrations [118]. Fig. 3 further revealed the relationship of IL hydrophobicity and the enzyme hydration [131]. The more hydrophobic of an IL (less negative of the $\log P$ value) is, the less water is required for maintaining optimal enzyme activity and enantioselectivity. When the IL is hydrophilic ($[BMIM][BF_4]$), more water presence is needed because high concentrations (high ionic strength) of hydrophilic ILs tend to remove the essential water from the enzyme causing the deactivation.

Other examples of the importance of water activity on biocatalysis in hydrophobic ILs include: a number of studies illustrated that water was required by α-chymotrypsin to main-

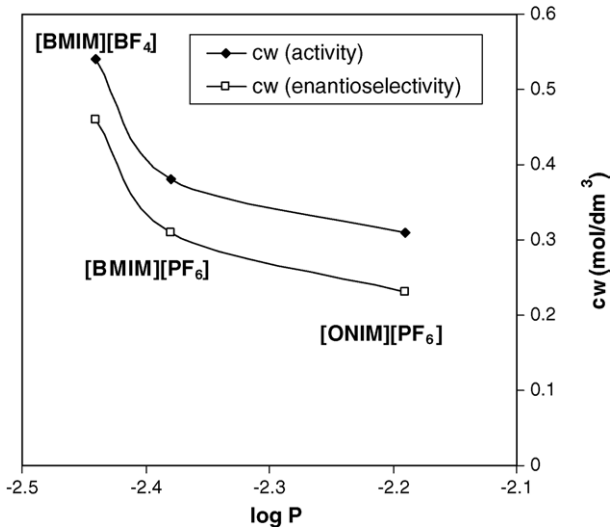

Fig. 3. The relationship of log P values of three ILs and the optimal hydration of *Candida rugosa* lipase {c_w (activity) is the water concentration for optimal hydration of the lipase activity; c_w (enantioselectivity) is the water concentration for optimal hydration of lipase enantioselectivity; ONIM is 1-octyl-3-nonylimidazolium} [131].

tain its enzymatic activity [123,132–134], and the bell-shaped relationship between reaction rates and water contents is similar to that in organic solvents; [132] Berberich et al. [135] used salt hydrate pairs to control the water activity for enzyme catalysis in ILs. Table 2 also demonstrated that higher enzyme activity might be observed in ILs with larger cations (such as $[OMIM]^+$) than those with smaller ones (such as $[BMIM]^+$). The longer hydrophobic alkyl chain in the cation has less tendency to take away the essential water from the enzyme [45]. The water-stripping ability of hydrophobic $[BMIM][(CF_3SO_2)_2N]$ was found comparable to that in polar organic solvents [134].

From the molecular level, the enzyme stabilization by water-immiscible ILs (such as $[(CF_3SO_2)_2N]^-$ types) was explained as a more compact enzyme confirmation (higher catalytic activity) formed from the evolution of α-helix to β-sheet secondary structure of the enzyme [136]. In conclusion, hydrophobic ILs (1) may strip off the essential water from the enzyme as organic solvents do [137]; (2) may interact with the enzyme through Coulombic (or electrostatic) interactions. (The Coulombic are known important factors for the stability of receptor–ligand complexes in aqueous environment [138–140]. The ion-pair formation is considered to release immobilized ions and water molecules resulting higher entropy, and is less favorable in concentrated solutions.) However, the inhibition mechanisms of enzymes by ions could not be explained entirely by the electrostatic interactions [53,57,80,84]; (3) may interact with substrates or products as organic solvents do [137].

On the other hand, with the presence of water in hydrophilic ILs, the effect of ions on the enzyme activity is related to the Hofmeister series (see the second part of Table 2) because these ILs dissociate into individual ions in water. Lau et al. [141] reported a high conversion in pure $[EMIM][BF_4]$ for the transesterification of ethyl butanoate with 1-butanol catalyzed by Novozym 435, but a much lower conversion in the same IL containing 10% (v/v) water. When water was involved, the chaotropic BF_4^- and kosmotropic $[BMIM]^+$ (explained later) could deactivate the enzyme. Our recent investigation [142,143] indicated that the protease stability in low concentrations of IL solutions could be related to the Hofmeister series (Table 2): kosmotropic anions (such as CH_3COO^- and CF_3COO^-) stabilize the enzyme while chaotropic ones (such as OTs^- and BF_4^-) destabilize it.

In the presence of water, the cations in ILs impose a considerable effect on the enzyme activity. Most data in the second part of Table 2 seem to support that enzymes exhibited higher activity and stability in ILs with smaller cations. The reason is because organic cations experience hydrophobic hydration [22,33,49–51], and smaller organic cations are chaotropic while larger ones are kosmotropic. It was well established [34,35,144–149] that larger tetraalkylammonium cations, such as $[n\text{-}Pr_4N]^+$ ($B = 0.916$), $[n\text{-}Bu_4N]^+$ ($B = 1.275$) and $[n\text{-}Pe_4N]^+$ (B unknown), are kosmotropes; smaller $[Et_4N]^+$ ($B = 0.385$) is a borderline ion and $[Me_4N]^+$ ($B = 0.123$) is a chaotrope. Based on Lowe and Rendall's data [150], we computed the B-coefficients of $[MePy]^+$ and $[EtPy]^+$ as 0.144 and 0.228, respectively, using $B = -0.073$ for I^- [25,35]. Lowe and Rendall also noticed that the B-coefficients of homologues are proportional to the number of carbon atoms. Based on this empirical relationship, we calculated the B-coefficient of $[BuPy]^+$ as ∼0.396 [25]. Comparing these B-values with those of tetraalkylammonium ions, $[MePy]^+$ and $[EtPy]^+$ are chaotropes while $[BuPy]^+$ is a borderline ion. The order of increasing kosmotropicity is $[MePy]^+ < [EtPy]^+ < [BuPy]^+$. All three cations could potentially stabilize the enzyme depending on their counter anions. The B-coefficients of imidazolium cations are not available, however, some preliminary thermodynamic results [151–153] seemed to indicate that $[EMIM]^+$ is a chaotrope and $[BMIM]^+$ is a kosmotrope based on their interactions with water molecules [25]. Larger imidazolium cations (such as $[HMIM]^+$ and $[OMIM]^+$) are expected to be kosmotropic. Our study confirmed that the protease exhibited higher stabilities in $[EMIM]^+$ based ILs than in $[BMIM]^+$ based ILs [142]. Turner et al. [154] noticed that the high Cl^- ion concentration did not seem to be the only reason for the inhibition of cellulase in [BMIM]Cl solution. Maybe the cation was relevant to the enzyme activity.

Besides regularly seen quaternary alkyl-substituted ammonium, phosphonium, imidazolium and pyridinium ILs, it is noticeable that new ILs based on pyrrolidinium cations (as well as triazolium, pyrazinium, pyridazinium, pyrimidinium, pyrazolium, piperazinium, thiazolium, oxazolium, oxazolidinium and morpholinium cations [155–157]) have recently been prepared, and their physical properties have been characterized [158–163]. As shown in Table 2, the Russell's group [130] reported higher stabilities of Novozym 435 in pyrrolidinium ($[MMEP]^+$) ILs than in $[BMIM]^+$ based ILs. Another example of using pyrrolidinium ILs for biocatalysis is the lipase-catalyzed synthesis of polyesters [164]. Although the B-coefficients and other hydration information of pyrrolidinium cations are not yet available, these new aliphatic ions are less toxic than aromatic

species [160] and thus are worthy of a further investigation as 'greener' media for biocatalysis.

Since cations and anions in ILs play equally important roles on the enzyme activity in aqueous environment, the customized combination of cations and anions will be crucial to the enzyme activity because a specific enzyme may require different LDW/HDW equilibrium as adjusted by the cation–anion pair. Kaftzik et al. [127] observed a higher activity of mandelate racemase from *Pseudomonas putida* in [MMIM][$MeSO_4$] solution than in [BMIM][$OctSO_4$] solution when the water activity is greater than 0.8. With the same water content, the water activity in [MMIM][$MeSO_4$] solution is always lower than that in [BMIM][$OctSO_4$]. It was suspected that $MeSO_4^-$ had stronger interactions with water molecules, and thus, is a kosmotrope. Therefore, the dual action of chaotropic cation and kosmotropic anion enabled a higher enzyme activity in the [MMIM][$MeSO_4$] solution. However, although mandelate racemase exhibited a low activity in low water content, its enantioselectivity was extremely high in various hydrophilic IL solutions (e.g., [MMIM][$MeSO_4$], [BMIM][BF_4], [BMIM][$OctSO_4$] and [PeMIM][BF_4]) [127].

5. Conclusions

The influence of inorganic salts on the enzyme activity and stability usually follows the Hofmeister series or the kosmotropicity order. The effect of ILs on the enzyme is more complicated especially when ILs are present as nearly anhydrous solvents. However, the enzyme stability seems to follow the Hofmeister series when the aqueous solutions of hydrophilic ILs are used as reaction media.

The enzyme activity in aqueous solutions is not solely determined by the ion kosmotropicity because the activity depends on the overall enzyme–medium–substrate relationship. For example, the substrate concentration might affect the enzyme inhibition by ions since the substrate could protect the enzyme against the inactivation [56]. Other factors of ILs (such as polarity [36], hydrogen-bond basicity [170,171] and anion nucleophilicity [130]), excipients (such as the support type of enzyme [132]), pH and impurities [37,45] also have considerable impact on the activity and stability of enzymes in ionic solvents.

The polarity of ILs has been extensively investigated using the solvatochromic analysis as a possible major factor for the enzyme activity. Many ILs have polarities around 0.6 on the Reichardt's polarity scale (0 for non-polar tetramethylsilane and 1 for polar water) [39,168]. Park and Kazlauskas correlated the enzyme (*Pseudomonas cepacia* lipase) activity with the IL polarity, indicating higher conversions achieved in more polar ILs [168]. However, many other studies [45,130,142,166] have not yet established a simple relationship between the enzyme activity and IL polarity.

More systematic studies of enzyme activity and stability in various ILs are needed in order to analyze the major factor that influences the enzyme behavior in these new organic solvents. More data of the ion kosmotropicity (such as *B*-coefficients) [25] are also needed for many organic cations.

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