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Effect of deep eutectic solvent mixtures on lipase activity and stability

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ABSTRACT

DESs (deep eutectic solvents) have many potential applications as cosolvents or anhydrous reaction media for biocatalytic reactions, owing to their non-volatility, non-flammability, non-toxicity, biocompatibility, biodegradability, and low cost. In this work, choline chloride ([Ch]Cl)-based DESs and DES mixtures containing two hydrogen bond donors were used as cosolvents to enhance the activity and stability of *Candida rugosa* lipase in aqueous reactions. The activity of lipase in an aqueous solution of [Ch]Cl:urea:glycerol was 155% higher than that in buffer. The half-life time of lipase at 40 °C in an aqueous solution of [Ch]Cl:glycerol was enhanced by 9.2 times. The lipase showed the highest acid stability and base stability in the aqueous solutions of [Ch]Cl:glycerol:thiourea and [Ch]Cl:ethylene glycol:formamide, respectively. In general, glycerol-containing DES mixtures were very useful in enhancing the activity and stability of lipase, while formamide-containing DES mixtures could not efficiently enhance the activity and stability of lipase. To understand the effect of DES mixtures on the activity and stability of lipase in aqueous solution, four solvatochromic parameters of DES mixtures were determined. When the solvatochromic parameters of DES mixtures were correlated with the stability of lipase in aqueous solutions of DES mixtures, it was found that thermal stability and storage stability of lipase were associated with the hydrogen bond acidity of DES mixtures. Acid stability and base stability of lipase were correlated with polarity based on Reichardt's dye and the dipolarity/polarizability of DES mixtures, respectively.

1. Introduction

Organic solvents have long been used as reaction media for non-aqueous enzyme reactions since the pioneering results of Klibanov [1]. Organic solvents as reaction media could greatly enhance the stability and selectivity of enzymes and could cause the shift of the chemical equilibrium. However nowadays, many studies are interested in the discovery of new reaction media for non-aqueous enzyme reactions because of the environmental toxicity, flammability, and volatility of organic solvents. From more than 10 years ago, many researchers to find alternative reaction media for enzyme reactions have focused on ionic liquids (ILs) [2,3]. ILs are organic salts that melt below 100 °C. Interest in ILs stems from their potential as "green solvents"; specifically, their non-volatile character and thermal stability make them attractive alternatives for volatile organic solvents [4,5]. It has been observed that the use of ILs enhances the reactivity, selectivity, and stability of enzymes [2–4,6,7]. Although ILs as reaction media have many advantages, the main limitations of ILs are their high cost, difficult synthesis, and purity requirement. In particular, the activity and stability of enzymes can be highly influenced by the impurities of ILs [8]. In addition, the green aspect of ILs is threatened nowadays because of their possible toxicity and low biodegradability [9].

Recently, to overcome the limitations of ILs, deep eutectic solvents (DESs) have been developed. DESs can be prepared simply by mixing an ammonium or phosphonium salt with a hydrogen bond donor (HBD), and purification of DESs is not required [10]. Choline chloride ([Ch]Cl) is the most frequently used cationic salt because it is biodegradable and costs less than US $960 per metric ton for 98% purity. Various HBDs such as alcohols, amides, amines, and sugars can be used to prepare DESs. The properties of DESs can be easily controlled by changing the mixing ratio of the cationic salt and HBD. The melting temperatures of some DESs are lower than room

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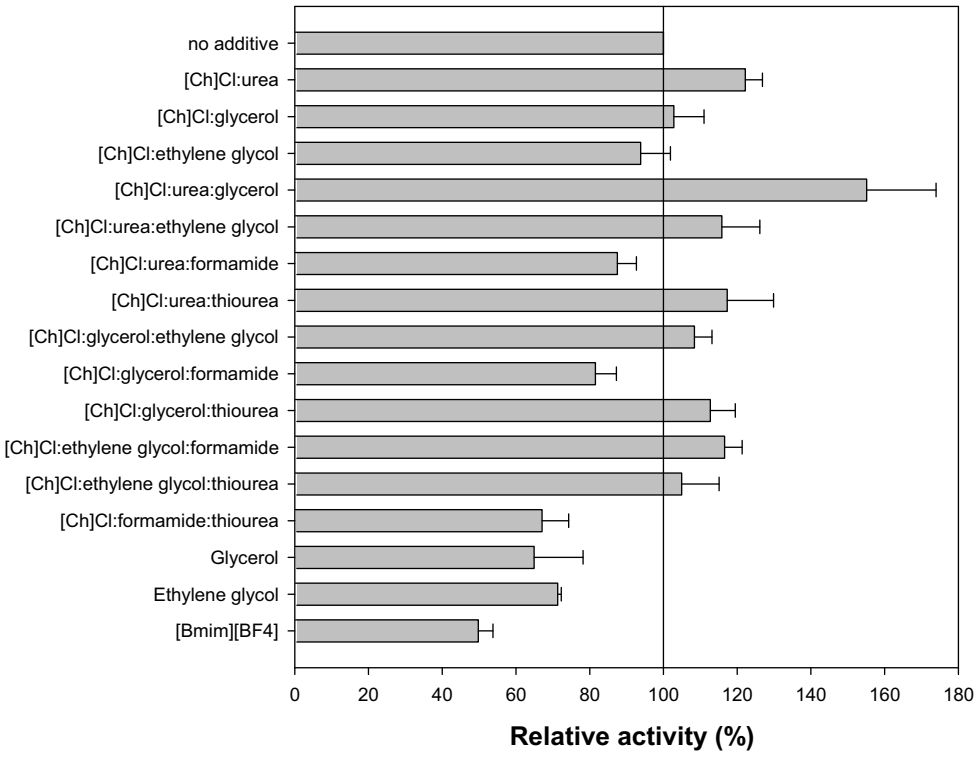

Fig. 1. Activity of lipase in aqueous solutions containing DES mixtures. The activity of lipase in buffer without additive was defined as 100%.

Table 1
Mixing ratios and water contents of the prepared DES mixtures.

DES mixture	Molar ratio	Water content (%)
[Ch]Cl:urea	1:2	1.65
[Ch]Cl:glycerol	1:2	0.91
[Ch]Cl:ethylene glycol	1:2	0.64
[Ch]Cl:urea:glycerol	1:1:1	0.59
[Ch]Cl:urea:ethylene glycol	1:1:1	0.63
[Ch]Cl:urea:formamide	1:1:1	0.61
[Ch]Cl:urea:thiourea	1:1:1	1.16
[Ch]Cl:glycerol:ethylene glycol	1:1:1	1.07
[Ch]Cl:glycerol:formamide	1:1:1	0.52
[Ch]Cl:glycerol:thiourea	1:1:1	1.16
[Ch]Cl:ethylene glycol:formamide	1:1:1	0.50
[Ch]Cl:ethylene glycol:thiourea	1:1:1	1.33
[Ch]Cl:formamide:thiourea	1:1:1	1.30

Table 2
Thermal stability of lipase in aqueous solutions containing DES mixtures.

DES mixture	Half-life time of lipase at 40 °C (h)
Buffer (no additive)	1.0
[Ch]Cl:urea	1.9
[Ch]Cl:glycerol	9.2
[Ch]Cl:ethylene glycol	5.3
[Ch]Cl:urea:glycerol	3.2
[Ch]Cl:urea:ethylene glycol	5.3
[Ch]Cl:urea:formamide	1.4
[Ch]Cl:urea:thiourea	2.0
[Ch]Cl:glycerol:ethylene glycol	8.9
[Ch]Cl:glycerol:formamide	5.0
[Ch]Cl:glycerol:thiourea	2.9
[Ch]Cl:ethylene glycol:formamide	6.1
[Ch]Cl:ethylene glycol:thiourea	6.0
[Ch]Cl:formamide:thiourea	0.9
Glycerol	5.3
Ethylene glycol	3.4

temperature. In general, DESs are cheap, easy to prepare, non-toxic, and biodegradable, while also providing the advantages of ILs such as non-volatility and high thermal stability. Therefore, the number of publications regarding the application of DESs is gradually increasing.

DESs as reaction media or cosolvents for biocatalysis have many more potential applications than organic solvents and ILs because of their non-toxicity, biocompatibility, biodegradability, and low cost. Recently, DESs have been used successfully as anhydrous reaction media for biocatalytic reactions. The activity, stability, and selectivity of enzymes such as lipase, protease, and epoxide hydrolase have been enhanced by using DESs as reaction media [9,11]. Gorke et al. studied lipase-catalyzed transesterification, aminolysis, and ring-opening polymerization in various DESs [12,13]. Zhao et al. showed that DESs based on cholinium salts and glycerol have merit as green solvents in lipase-catalyzed transesterification and preparation of biodiesel [14,15]. Durand et al. investigated the CALB-catalyzed alcoholysis of vinyl laurate [16].

Studies on the use of DESs as anhydrous reaction media for enzyme-catalyzed reactions have been gradually increasing, while very few results regarding the use of DESs as cosolvents for enzyme reactions have been reported. Gorke et al. reported that the conversion of styrene oxide catalyzed by epoxide hydrolase from *Agrobacterium radiobacter* was enhanced up to 20-fold in the presence of 25% [Ch]Cl:glycerol [12]. Lindburg et al. investigated the effect of DESs on the potato epoxide hydrolase-catalyzed hydrolysis of (1,2)-*trans*-2-methylstyrene oxide [17]. Huang et al. used cholinium-salt-based DESs as cosolvents for *Penicillium expansum* lipase (PEL) in aqueous solution [18]. The activity and stability of PEL could be increased up to 1.4-fold and 17.4-fold, respectively, by using [Ch]Ac:glycerol. Wu et al. used cholinium-salt-based DESs as cosolvents for horseradish peroxidase (HRP) in aqueous solution [19]. They reported that [Ch]Cl-based DESs have been found to be superior to the [Ch]Ac-based DESs in terms of promoting HRP activity. Although some DESs have proved to be good cosolvents for biocatalysis, the effect of DESs on the activity and stability of