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Pure and aqueous deep eutectic solvents for a lipase-catalysed hydrolysis reaction

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ABSTRACT

This study presents the potential role of cholinium-based deep eutectic solvents (DESs) in a lipase-catalysed hydrolysis reaction as both a co-solvent in an aqueous solution and as a main solvent. The hydrolysis of *para*-nitrophenyl palmitate by *Burkholderia cepacia* lipase (BCL) was selected as the model reaction. For comparison purposes, the tested DESs were studied with 1-ethyl-3-methylimidazolium tetrafluoroborate and methanol were used as representatives of ionic liquids and organic solvents, respectively. The results showed that choline chloride: ethylene glycol (ChCl:EG) was the most promising solvent among the tested DESs. In the presence of 40% v/v of ChCl:EG in buffer medium, lipase activity was enhanced by up to 230% compared to a free-DESs system. In contrast, the enzyme was mostly deactivated in pure DESs. However, adding 4% v/v of water to a pure DES medium increased enzyme activity by up to 2.6 times over that in a phosphate buffer, 1.5 times higher than in IL and 14 times higher than in methanol. Kinetic analysis showed that enhancement of enzymatic efficiency in the DES system was due to an increase in enzyme affinity towards the substrate.

1. Introduction

Organic solvents such as hexane and methanol are the main media used for enzymatic biotransformations. Despite the significant improvement in enzyme activity using organic solvents, they are also considered environmentally toxic, flammable, and enzyme destructive [1]. As a result, ionic liquids (ILs) have emerged as a potential replacement for organic solvents [2]. Researchers have shown that biocatalyst activity, thermal stability, and reusability can all be improved in ILs [3,4]. However, due to the high cost and complex synthesis, ILs as a replacement for organic solvents are currently not viable for industrial application.

Recently, DESs have emerged as promising environmentally-friendly solvents. These solvents gained widespread attention and curiosity from the academic and industrial research communities. DESs as ILs analogous are composed of a salt and hydrogen bond donor (HBD) that form a eutectic mixture with a much lower melting point than any of the individual components [5]. Just like ILs, DESs have high thermal stability, low volatility, and the melting point is often close to room temperature [6]. Several publications reported that DESs possess low toxicity and high biodegradability [7–9]. They are also less expensive than ILs and easier to prepare [10]. Therefore, DESs have been utilized in a number of applications, including as solvents for the electrodeposition of metals [11], nanotechnology catalyst [12], or as media for several chemical and biochemical reactions [5,13].

Recent advances in DESs have inspired many researchers to exploit them as reaction media for biocatalysis. They have shown promising results as solvents or co-solvents in a wide range of biotransformation reactions [6,14]. The reactions studied so far involved lipase, protease and cellulose-catalysed reactions, such as: transesterification [15], hydrolysis [16], alcoholysis [6] and other conversion reactions. To date, lipases-catalysed reactions are the most studied enzymes and they maintained high activity in DESs [17]. In 2008, Gorke et al. [16] studied the transesterification of ethyl valerate assisted by different ChCl-based DESs. Other studies found that glycerol as a HBD coupled with different salts in DESs had better compatibility with both lipase and protease enzymes [14,17,18]. Huang et al. [19] found that *Penicillium expansum* lipase was influenced by the selection of DES components and ratio of the composition. To date, examinations in DESs have been confined mainly for lipases catalysed reactions of esterification, transesterification, alcoholysis or acidolysis [6,17,20]. Moreover, the use of

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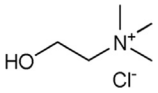
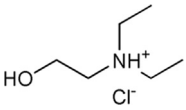
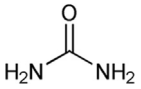
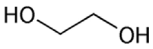
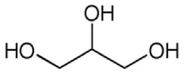
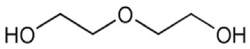
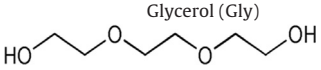
Table 1
Structures, names, and abbreviations of the individual components of the tested DESs.

Ammonium salts	
 Choline chloride (ChCl)	 N,N-diethyl ethanol ammonium chloride (EAC)
Hydrogen bond donors	
 Urea (U)	 Ethylene Glycol (EG)
 Glycerol (Gly)	 Diethylene Glycol (DEG)
 Triethylene Glycol (TEG)	

DESs as the main solvent was limited to only nonaqueous solution reactions.

In this work, we examined the feasibility of using DESs as both a co-solvent and as a main solvent for amano lipase PS from *Burkholderia cepacia* (BCL). The aim was to study the activity and stability of the enzyme with several types of DESs and to optimize reaction conditions such as water content, temperature, and reaction time. The effect of DESs on the enzyme kinetics represented by K_M and K_{cat} was also examined. The tested DESs were prepared by a combination of two salts and five HBDs as illustrated in Table 1. The selection was based on the most commonly DESs well characterized and used [14,19].

Methanol and 1-ethyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4] were tested as a representative organic solvent and IL, respectively. Both were selected based on their frequent usage in previous studies with enzymes and their similar physical properties with the tested DESs. Methanol has been previously tested with different types of enzymes such as Horseradish peroxidase, penicillin acylase and *E. coli* penicillin amidase [21–24]. The polarity, according to the colour of dissolved Reichardt's dye for all the examined DESs falls between E^T_N 0.77–0.90 [16]. In addition, the polarity of methanol at 0.76 and [BMIM][BF_4] at 0.68 [25] is within the range. It is noteworthy that methanol and [BMIM][BF_4] have high solubility in water which make them suitable solvents for comparison with DESs [26].

2. Materials and methods

2.1. Biological and chemical materials

Amano Lipase PS was utilized from *Burkholderia cepacia* (BCL), with DESs components ChCl (purity ≥ 98%), U (purity 99.5%) and Gly (purity 99%). *p-Nitrophenyl palmitate*, *p*-nitrophenol, isopropanol and Sodium deoxycholate (purity ≥9 7%) were purchased from Sigma-Aldrich, while EAC (purity ≥ 98%), EG (purity 99%), DEG (purity 99%), TEG (purity 99%), ethanol (purity ≥ 99.9%) and 1-ethyl-3-methylimidazolium tetrafluoroborate [EMIM][BF_4] (purity 98%) were purchased from Merck.

2.2. DESs preparation

A cholinium salt (ChCl or EAC) and a HBD were mixed at a specific molar ratio as indicated in Table 2 in pre-sterilized and dark flasks. The flasks were then heated and stirred in a shaker incubator at 70 °C until a colorless liquid formed after almost 2 h [19]. The water content of all DESs ranged from 0.43–1.23% as determined by Karl-Fischer titration.

Table 2
Compositions and molar ratios of the tested DESs.

DES composition	Molar ratio
ChCl:Gly	1:2
ChCl:U	1:2
ChCl:EG	1:2
ChCl:DEG	1:2
EAC:Gly	1:2
EAC:EG	1:2
EAC:TEG	1:2

2.3. DESs as co-solvent and main solvent

When DESs was used as co-solvent, BCL powder of 1 mg was diluted in different amounts (0.3, 0.6, 0.9, 1.2 ml) for each solvent (DESs, IL, and methanol) for 20 min and mixed gently. Then, the mixtures were used directly to make 10, 20, 30, and 40% (v/v), respectively, of the total volume of the aqueous reaction media. In the second part of the study, DESs were the main solvent for both substrate and enzyme without any other co-solvents. BCL powder (1 mg) was diluted into 0.6 ml of DESs to form a lipase/DES mixture. The reaction was then started by adding the lipase/DES mixture to the substrate/DES mixture.

2.4. Enzyme activity assay

2.4.1. In aqueous solution (DESs as co-solvent)

Lipase enzyme activity in aqueous solutions was determined spectrophotometerically. Hydrolysis of *p*NPP to *p*NP was selected as a standard reaction to determine lipase activity. The same method described in previous studies [19,27] was followed with a slight modification. The enzyme substrate was prepared accordingly (30 mg of *p*NPP was diluted in 10 ml of isopropanol) and mixed with 90 ml of the phosphate buffer (pH 8.0). The buffer was made out of potassium dihydrogen phosphate, 207 mg of sodium deoxycholate, 100 mg of Arabic gum, and 1.8 ml of Triton X-100 to remove turbidity. The final mixture was gently mixed and warmed at 37 °C. The reaction was started by adding the pre-treated lipase/DESs mixture to 3 ml of freshly prepared 3.0 mM substrate in the phosphate buffer solution inside 10 ml tubes. The tubes were incubated in a water bath at a selected temperature for 5 min before the reaction was stopped by adding 1 ml 0.1 M NaOH to the reaction mixture [28]. The absorption spectra of *p*NP were monitored using a PerkinElmer-Lambda UV–vis spectrophotometer at 410 nm. Each assay was replicated thrice for each DES and the results were presented as mean of the replicate assay ± standard deviation (SD).