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Novozym 435 catalyzed regioselective acylation of ethane-1,2-diol in the presence of ionic liquids

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The acylation of ethane-1,2-diol catalyzed by lipase (Novozym 435) with ethyl acetate in 1-butyl-3-methyl imidazolium hexaflorophosphate [BMIM][PF_6] and 1-butyl-3-methyl imidazolium tetrafloroborate [BMIM][BF_4] was studied. It was demonstrated that the activity of the enzyme in [BMIM][BF_4] was very low for this reaction, while it was very active in [BMIM][PF_6]. Both the equilibrium conversion and the selectivity to ethane-1,2-diol monoacetate (EDMA) in [BMIM][PF_6] could be considerably higher than that at solvent-free condition. The IL and enzyme have synergetic effect to prevent thermal deactivation of the enzyme. Addition of small amount of water in [BMIM][PF_6] could enhance the conversion and selectivity to EDMA.

KEY WORDS: ionic liquids; Novozym 435; ethane-1; 2-diol; transesterification.

1. Introduction

Room temperature ionic liquids (ILs) have received much attention in recent years [1]. They consist of only ions and are liquids at or close to room temperature, have no detectable vapor pressure, and are thermally stable. In addition, ILs possess widely tunable properties with regard to polarity, hydrophobicity, and solvent miscibility behavior through appropriate modifications of the cations and anions. Enzymes, especially lipases, have been successfully used in ILs. Besides environmental benefit, lipase catalyzed reactions in ILs showed better enzyme stability and product enantioselectivity [2,3]. Nara *et al.* [4] conducted the lipase catalyzed transesterification reaction between 2-hydroxymethyl-1,4-benzodioxane and vinyl acetate in the presence of 1-butyl-3-methyl imidazolium hexaflorophosphate [BMIM][PF_6] and 1-butyl-3-methyl imidazolium tetrafloroborate[BMIM][BF_4]. The catalytic activity in [BMIM][PF_6] was comparable or better than that in organic solvents, while that in [BMIM][BF_4] was relatively poor. Enantio- and regio- selectivity of the products could be improved in the presence of ILs [5], and some ILs were proved to be much less toxic for whole cell biocatalyst than organic solvents [6]. Additionally, ILs are good stabilizing agents, which prevents thermal deactivation of the enzymes [7]. Many ILs appear to be cleaner alternatives to organic solvents for biocatalysis. Furthermore, application of ILs is advantageous as they can be reused together with the enzyme with durable enzymatic activity [8]. On the other hand, some of the ILs themselves have been proved to be catalyst for a number of chemical reactions. Duan *et al.* [9] conducted esterification reaction of tertiary butanol and acetic anhydride in [BMIM][BF_4] with excellent yield and selectivity.

Regio-selective acylation of polyhydroxy compounds has been an interesting topic for many years. Selective acylation can be done with different conventional chemical methods. Different investigators have used lipases in ILs for selective acylation of compounds possessing more than one hydroxyl groups present at different positions in a ring containing carbon and oxygen atoms [10–12]. To the best of our knowledge, there is no report about acylation of hydroxyl groups present at similar positions in a carbon chain.

The aim of the present work is to use enzyme-IL couple to enhance the selectivity of mono acylated product from compounds having more than one similar hydroxyl groups. In this connection we carried out the lipase-catalyzed acylation of ethane-1,2-diol (ED) in [BMIM][PF_6] and [BMIM][BF_4] with ethyl acetate (EA) as acylating agent (Scheme 1). It was demonstrated that the presence of [BMIM][PF_6] in the reaction medium selectively produced ethane-1,2-diol monoacetate (EDMA) as major product with high conversion. The reaction was also conducted at solvent-free condition for comparison.

2. Experimental

Ethylene glycol, Ethyl acetate, having purities of 96% and 99.5%, respectively, were obtained from Beijing Chemical Reagent Plant and were used without further purification. Lipase (Novozym 435) was purchased from Novozymes in Beijing. The ILs, [BMIM][PF_6] and

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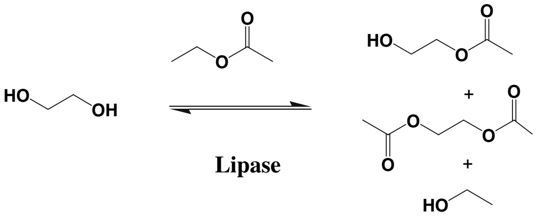

Scheme 1. Lipase catalyzed acylation of ethane-1,2-diol.

[BMIM][BF_4], were synthesized using the procedures reported by other authors [13].

Reactions were carried out in glass vials. After specific time interval 0.1 mL of the sample was taken from the reaction vial and extracted with benzene. Known weight of toluene was added as internal standard. Samples were analyzed by Gas Chromatograph (Agilent 4890D) with N_2 as the carrier gas. Conversion was calculated as the percentage of number of moles of ED converted to the products, and the selectivity was determined as the percentage of number of moles of EDMA and EDDA formed in the total number of moles of the two species.

3. Results and discussion

3.1. Effect of reaction time on the reaction

Hydrophobic and hydrophilic nature of the above mentioned ILs is due to the attachment of different anions to the same organic cation. This feature is very important in enzyme catalyzed reactions as it influences the conformation of enzyme and hence its activity [5]. The conversion in [BMIM][PF_6] was much higher than that in [BMIM][BF_4], as shown in figure 1. The difference in conversion was partly related to the fact that the substrate is completely soluble in former and insoluble in the latter. Moreover, [BMIM][PF_6] is hydrophobic and favored the enzyme action by enhancement of free water molecule which acted as a nucleophilic reagent to increase the conversion [14], while the hydrophilic nature of [BMIM][BF_4] might allow it to extract the water molecule from the enzyme microenvironment, making it unfavorable for enzyme action [4]. On the other hand, figure 2 demonstrates that in [BMIM][PF_6] the selectivity to EDMA was greater than that at solvent free condition, indicating that the presence of the solvent favored the formation of EDMA. After checking the suitability of [BMIM][PF_6] for the transesterification reaction the subsequent experiments were conducted in [BMIM][PF_6] as the solvent and at solvent-free condition.

3.2. Reaction at solvent-free condition

The effect of enzyme concentration on the conversion without any solvent is shown in figure 3. Conversion increased with increase of enzyme concentration in lower concentration range, while the conversion was nearly independent of enzyme concentration in the high concentration range. The main reason is that as enzyme concentration was low there were less active sites of the enzyme, and reaction equilibrium could not be reached in the limited reaction time (70 min). However, there were enough active sites for the reaction to reach equilibrium as the concentration of the enzyme exceeded certain values. Therefore, further increase in enzyme concentration could not increase the conversion. Figure 4 demonstrates the dependence of the selectivity of the reaction on the enzyme concentration at solvent-free condition. At the lower enzyme concentration the selectivity was sensitive to enzyme concentration, and the ratio of EDMA/EDDA reduced greatly with

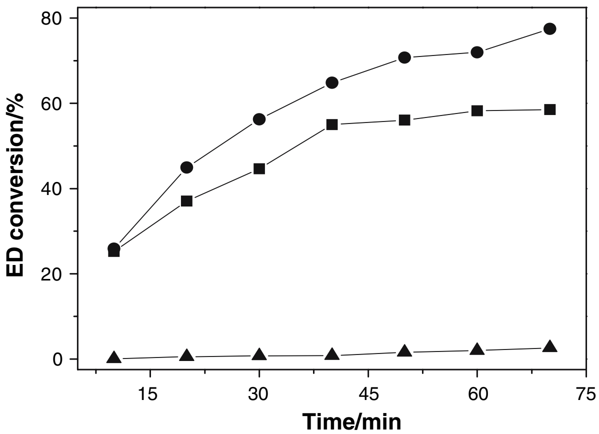

Figure 1. Effect of time on the conversion of ED ▲ [BMIM][BF_4]; ● [BMIM][PF_6]; ■ solvent-free; (ED: EA mole ratio 1:3, lipase 7 g /mole of ED, [BMIM][PF_6] 23.67 g/mole of ED, [BMIM][BF_4] 18.4 g/mole of ED, 40 °C).

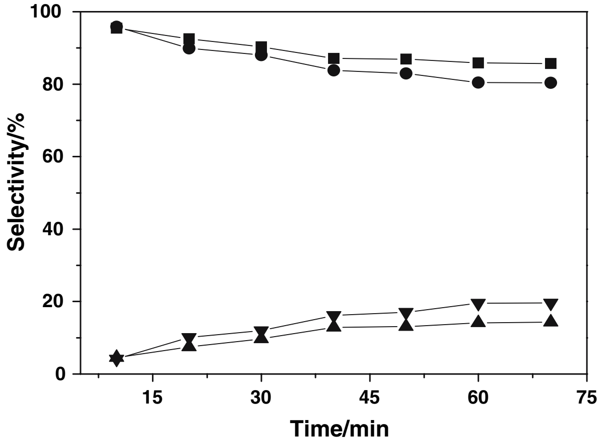

Figure 2. Effect of time to the selectivity of EDMA and EDDA ▲ Selectivity to EDDA in [BMIM][PF_6] and ▼ solvent free conditions; ■ selectivity of EDMA in [BMIM][PF_6] and ● solvent free conditions; (ED: EA mole ratio 1:3, lipase 7 g/mol of ED, [BMIM][PF_6] 23.67 g/mol of ED, 40 °C).

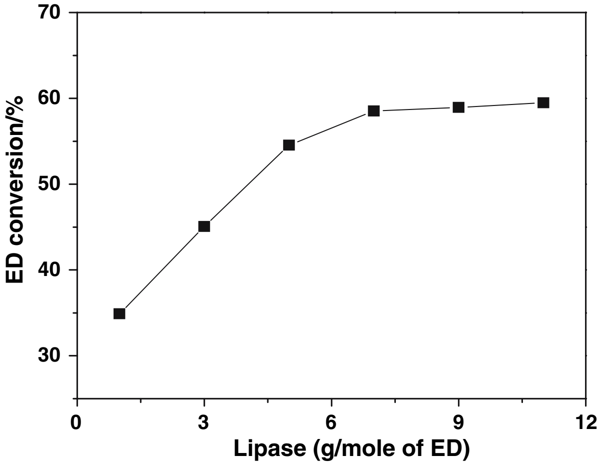

Figure 3. Effect of lipase concentration on the conversion of ED in the absence of solvent (ED: EA mole ratio 1:3, 40 °C, 70 min).

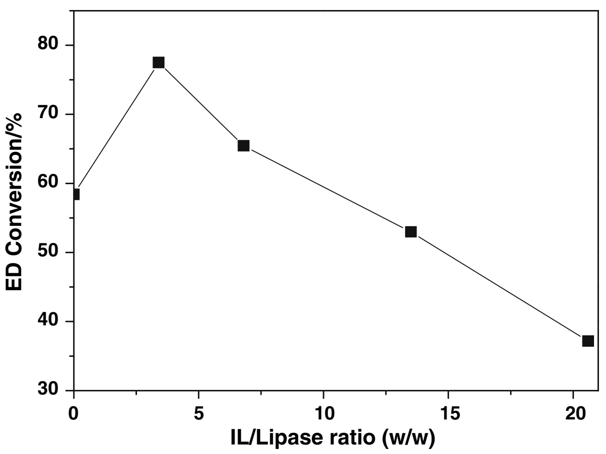

Figure 5. Effect of IL/lipase ratio (w/w) on ED conversion (ED: EA mole ratio 1:3, lipase 7 g/mol of ED, 40 °C, 70 min).

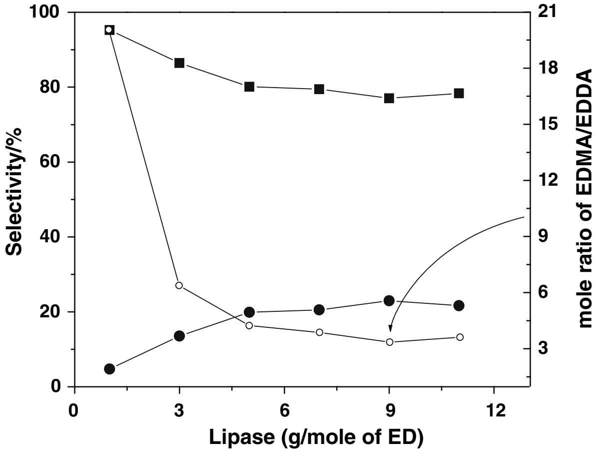

Figure 4. Effect of lipase concentration to the selectivity of EDMA and EDDA in the absence of solvent ; ● EDDA; ■ EDMA; ○ mole ratio of EDMA/EDDA (ED: EA mole ratio 1:3, 40 °C, 70 min).

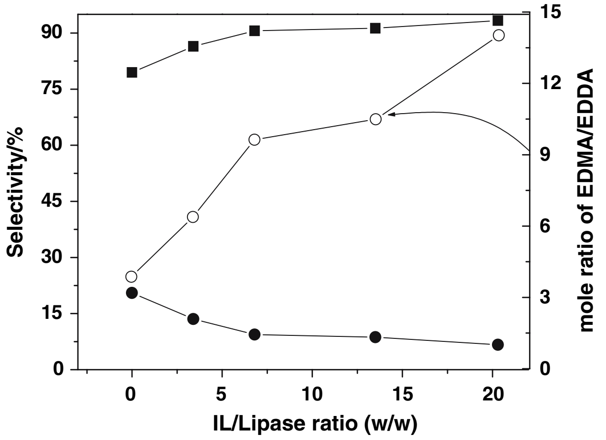

Figure 6. Effect of IL/lipase ratio (w/w) on the selectivity of EDMA and EDDA ● EDDA; ■ EDMA; ○ mole ratio of EDMA/EDDA (ED: EA mole ratio 1:3, lipase 7 g/mol of ED, 40 °C, 70 min).

increasing enzyme concentration. The selectivity was nearly constant at higher enzyme concentration.

3.3. Reaction in [BMIM][PF_6]

The IL/lipase ratio also played an important role for the conversion. It was observed that addition of IL up to certain limit increased the conversion, while further increase in IL concentration led to decrease in conversion, as illustrated in figure 5. However, the ratio of EDMA/EDDA increased continuously with increasing IL/lipase ratio (figure 6). It results mainly from the fact that at higher IL/lipase ratio the enzyme and reactants were diluted by the IL, and therefore the opportunity of the reactants to contact the enzyme became smaller.

Comparing the data in figures 3 and 6, it can be known that to get pure EDMA selectively is difficult. However, by optimizing experimental conditions both the conversion and the ratio of EDMA/EDDA in [BMIM][PF_6] could be considerably higher than that at solvent-free condition.

3.4. *Effect of water on the reaction*

Our experiments demonstrated that small amount of water in the reaction system increased the specific activity of the enzyme, and thus increased the conversion and selectivity of the products as illustrated in table 1. However, addition of excess of water decreased the conversion. The main reason is that excess water can lead to hydrolysis reaction and decreased conversion [15], which was confirmed by the fact that the intensity of the peaks of acetic acid in GC spectra increased with increasing water concentration, as was observed in our experiments. On the other hand, increase in water concentration continued to increase the EDMA/EDDA ratio (figure 7), producing almost pure EDMA. Thus water and the IL played an important role to enhance the selectivity.

Table 1
Effect of added water on the conversion of ED[a]

Water(μL)	Conversion (%)
00	76.3
10	81.7
50	75.0
100	73.9
150	73.7
200	72.0

[a] ED 0.003 mole, EA 0.009 mole, Lipase 7 g/mole of ED, [BMIM][PF$_6$] 23.67 g/mole of ED, 40 °C, 70 min.

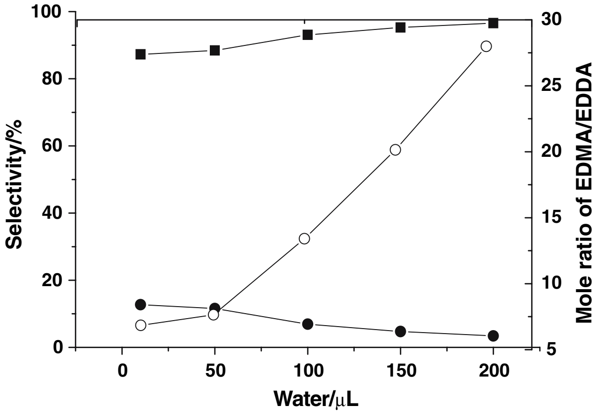

Figure 7. Effect of the amount of water added on the selectivity of EDMA and EDDA ● EDDA; ■ EDMA; ○ mole ratio of EDMA/EDDA (ED 0.003 mole, EA 0.009 mole, lipase 7 g/mol of ED, [BMIM][PF$_6$] 23.67 g/mol of ED, 40°C, 70 min).

Table 2
Effect of temperature on conversion of ED and selectivity of EDMA and EDDA in [BMIM][PF$_6$][a]

T/°C	Conversion (%)	Selectivity (%)	
		EDMA	EDDA
40	76.0	85.3	14.7
50	78.5	84.8	15.2
60	86.0	85.3	14.8

[a] ED: EA mole ratio 1:3, Lipase 7 g/mol of ED, [BMIM][PF$_6$] 23.67 g/mol of ED, 70 min.

3.5. *Effect of temperature on the reaction*

The conversion and selectivity of the reactions at 40 °C, 50 °C, and 60 °C are given in table 2. The conversion increased with increasing temperature, and the EDMA/EDDA ratio was nearly independent of temperature. Our previous work indicated that the effect of temperature on conversion was not considerable in the absence of IL [16]. This hints that the IL and enzyme have synergetic effect to prevent thermal deactivation of the enzyme.

4. Conclusion

Lipase (Novozym 435) is very active for the acylation of ethane-1,2-diol in [BMIM][PF$_6$]. At suitable condition, the equilibrium conversion of ED and the selectivity to EDMA in [BMIM][PF$_6$] can be considerably higher than that at solvent-free condition. The IL and enzyme have synergetic effect to prevent thermal deactivation of the enzyme, and addition of small amount of water in [BMIM][PF$_6$] can enhance the conversion of ED and selectivity to EDMA.

Acknowledgements

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