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Markedly enhancing lipase-catalyzed synthesis of nucleoside drugs' ester by using a mixture system containing organic solvents and ionic liquid

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Abstract—Eightfold higher yields and three times faster reaction rates were achieved by means of using a mixture solvent system composed of 90% acetone and 10% [BMIM]BF_4 in the lipase-catalyzed regioselective synthesis of polymerizable ester of nucleoside drugs.
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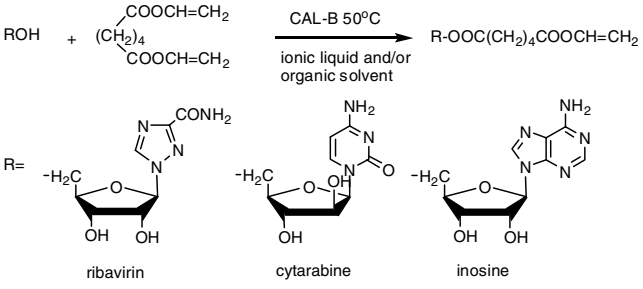
Nucleoside analogues have been widely applied for cancer and viral chemotherapy,[1] which represents an important research area for drug discovery. One of the most important strategies to synthesize their derivatives is the modification of the sugar moiety, which can improve bioavailability, reduce adverse effects, and increase antiviral activity.[2] However, it is difficult to selectively modify nucleoside analogues due to the multifunctional groups' nature of these molecules.

Regioselective biocatalysis has played an important role in the development of pharmaceutical compounds such as nucleoside analogues and natural products.[3] As a result, the enzymes can be recruited to modify nucleoside analogues under mild conditions without the need of tedious steps.[4] However, the reaction rate and yield of enzymatic reactions were usually much slower and lower. Therefore, it is desirable to develop new strategies to improve the activity of enzymes. Several methods have been reported to enhance the activity or selectivity of enzymes in a nonaqueous solvent system, and adding proper additives was one of the most simple methods.[5]

Ionic liquids (ILs)[6] are known to be 'green' alternatives to common solvents because they have no measurable vapor pressure compared with molecular organic solvents, and they are good solvents for many compounds, especially the polar ones. It is reported that ionic liquids would improve the stability and activity of enzymes.[7] Drauz et al. used ionic liquids as additives in the synthesis of chiral disubstituted malonates.[8] And Bornscheuer and Ganske applied a mixture composed of ionic liquid and 40% *tert*-butanol to synthesize glucose fatty acid ester in good yield.[9]

Herein, we would like to report using ionic liquids as additive in the lipase-catalyzed nucleoside drugs' ester synthesis reactions, and faster reaction rate and higher yield were achieved.

The ribavirin was acylated by divinyladipate (Scheme 1) catalyzed by lipase acrylic resin from *Candida antarctica* (CAL-B).[10a] The starting point of the present work was to identify an appropriate ratio of ionic liquid as



Scheme 1. Enzymatic regioselective synthesis of vinyl ribavirin ester, vinyl cytarabine esters, and vinyl inosine esters.

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additive. The amount of $[BMIM]BF_4$[11] in acetone was progressively increased from 0 to 100% (v/v). The result (Fig. 1) showed that there was no reaction when the reaction media contained $[BMIM]BF_4$ more than 60%. High concentrations of IL cause high ionic strength in the reaction media that might partially inactivate the enzyme. Also, the high viscosity of the reaction mixture caused by high concentrations of IL may limit the mass transfer of the substrate and product to and from the active sites of the enzyme, and may contribute to the fall in the yield at high IL concentrations. While, there was a clear contrast in the reaction. A remarkable enhanced yield was accomplished when the reaction was carried out in a solvent system composed of 90% acetone and 10% $[BMIM]BF_4$ (mixture).

Consequentially, CAL-B was used to catalyze the transesterification of ribavirin in the mixtures which were composed of different kinds of organic solvents and $[BMIM]BF_4$. And its activity was determined in terms of the extent of esterification. From Table 1, the yield was up to 98% in acetone (Entry 2) and moderate in dioxane (Entry 1). However, poor results were obtained in THF and dichloromethane (Entry 3, 4). No product was detected when *tert*-pentylalcohol, toluene or *n*-hexane was used (Entries 5, 6, and 7). It showed that the mixture solvent composed of acetone and $[BMIM]BF_4$ was more suitable for CAL-B in this fixed ratio. We also investigated other kinds of ionic liquids as additives as shown in Figure 2. Yield decreased drastically when the cationic part and anionic part were replaced which conformed ionic part affecting lipase. It was agreed with that the lipase activity was dependent on the combination of both parts of the imidazolium salts.[12]

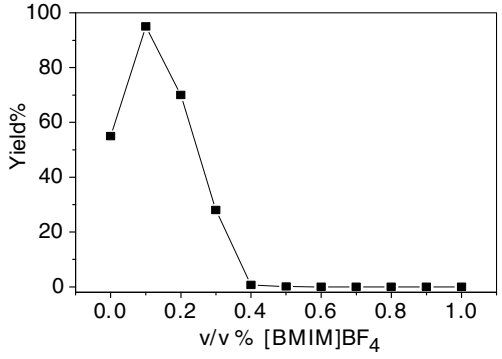

Figure 1. Ribavirin transesterification catalyzed by CAL-B in mixture solvents composed of the different ratios of acetone to $[BMIM]BF_4$ at 50 °C.

Table 1. Ribavirin transesterification catalyzed by CAL-B in mixture solvents composed of $[BMIM]BF_4$ and different organic solvents

Entry	10% (v/v) additive	Organic solvent	Yield[a] (%)
1	$[BMIM]BF_4$	Dioxane	21.3
2	$[BMIM]BF_4$	Acetone	98.5
3	$[BMIM]BF_4$	THF	0.6
4	$[BMIM]BF_4$	Dichloromethane	5.2
5	$[BMIM]BF_4$	*tert*-Pentylalcohol	n.d[b]
6	$[BMIM]BF_4$	Toluene	n.d.
7	$[BMIM]BF_4$	*n*-Hexane	n.d.

[a] Reacted 12 h at 50 °C; determined by HPLC.
[b] not detected.

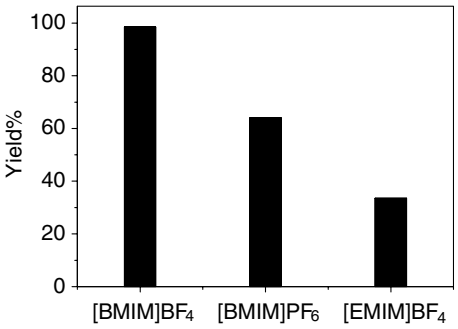

Figure 2. Ribavirin transesterification catalyzed by CAL-B in mixture solvents composed of 10% different ionic liquids and acetone at 50 °C.

A comparative study on the extent of the ribavirin's transesterification as a function of time in mixture as well as in acetone was carried out.[10b] The initial rate of transesterification dramatically increased three times from 0.41 mmol ml^{-1} h^{-1} in acetone to 1.44 mmol ml^{-1} h^{-1} in mixture (Fig. 3). The reaction time in mixture was only half of that in acetone to complete the reaction. It is assumed that two factors may be involved in the improvement of the enzymatic reaction. One is that a small percent of $[BMIM]BF_4$ would increase the solubility of nucleoside drugs because of its highly polar nature. To corroborate this point, the solubility in different media was determined. The result showed that ribavirin dissolved 13 mg ml^{-1} in mixture, nearly double of that in acetone (7 mg ml^{-1}). The other is the modified flexibility of the enzyme by ionic liquid. It was established that the change of the flexibility of lipase protein would reflect the performance of the enzyme.[13] Ionic liquid has a certain impact on the flexibility of the lipase through the electrostatic attractive interactions of the anion form of the ionic liquids with the cation of the amino residues.[14]

Other nucleoside drugs including cytarabine and inosine were tested (Scheme 1), because the activity of CAL-B in

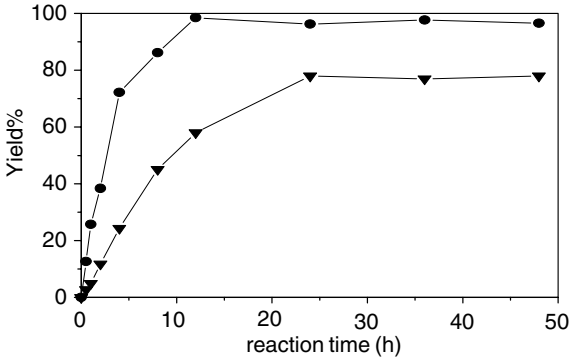

Figure 3. Time course of the ribavirin transesterification catalyzed by CAL-B in acetone (▼) and in co-solvent (●) at 50 °C.

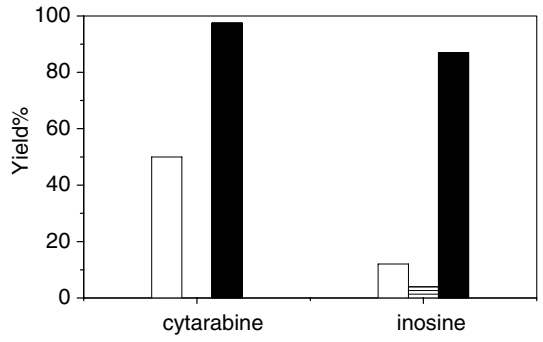

Figure 4. Comparison of the transesterification of cytarabine and inosine catalyzed by CAL-B in acetone (□), in [BMIM]BF_4 (▤), and in co-solvent (■) at 50 °C.

the acylation of ribavirin was enhanced markedly. Both of them were acylated regioselectively at primary hydroxyl group based on our previous works.[4] The yields (Fig. 4) of the acylation of cytarabine and inosine in the mixture system were increased twofold and eightfold of that in acetone, respectively.

In conclusion, the environmentally benign ionic liquid [BMIM]BF_4 is found to be a suitable additive for the enzymatic reactions of nucleoside drugs. Acylation of the nucleoside drugs was carried out in the mixture with the advantages of excellent regioselectivity, rapid reaction rate, and high yield.

Acknowledgment

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10. (a) In a typical procedure, the reaction was initiated by adding 5 mg CAL-B to 10 ml vessel containing 1 ml mixture, 0.05 mmol nucleoside analogues, and 0.2 mmol divinyladipate. The suspension was kept at 50 °C and shaken at 200 rpm. Samples were taken from the reaction mixture and determined by HPLC with a UV–vis detector and a reversed-phase Hypersil ODS2 column (250 × 4.6 mm). Elution was performed with a mixture of methanol/water (40/60, v/v) at 1 ml min^{-1}.; (b) Fifty milligrams of CAL-B was added to 50 ml vessel containing 20 ml mixture, 0.2 g (1 mmol) nucleoside analogues, and 4 mmol divinyladipate. Samples were taken from the reaction mixture after fixed intervals of time and determined by HPLC.
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