N-Methylimidazole significantly improves lipase-catalysed acylation of ribavirin

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_N_-Methylimidazole, a molecular solvent, but also, in cationic form, a component of 1-alkyl-3-methylimidazolium ($[C_nMIM]^+$) ionic liquids, showed promise as an additive in accelerating remarkably transesterification catalyzed by lipase acrylic resin from *Candida antarctica* (CAL-B).

The original work by Zaks and Klibanov in the early 1980s demonstrated that many enzymes maintain their activity in hydrophobic organic solvents.[1] A major advantage of applying enzymes in organic media is to avoid hydrolysis when performing non-hydrolytic transformations, such as the acylation of alcohols and amines, which are now major industrial applications.[2] A serious obstacle of conventional organic media is, however, the substantial reduction in reaction rate. Therefore, it is desirable to develop new strategies to shorten the time of enzymatic transformations. Several methods have been reported to enhance the activity or selectivity of enzymes in non-aqueous solvent systems, and adding suitable additives is among the most simple of methods.[3] There are, however, environmental drawbacks with polyethylene glycol (PEG) or sodium dodecyl sulfate (SDS) which are usually used in aqueous solution as additives.[4]

Ionic liquids are emerging as 'green' alternatives to common solvents because they have no measurable vapor pressure and are able to dissolve compounds of varying polarity. Enzyme catalysis in ionic liquids therefore is expected as a unique process leading to an environmentally benign chemistry. The pioneering work by Erbeldinger *et al.* and Sheldon and co-workers has triggered an exploration of potential benefits of enzyme catalysis in ionic liquids.[5] Recently, several research groups have reported the use of ionic liquids as efficient additives for biocatalytic processes. In separate work, the groups of Itoh and Zong added a small percentage of ionic liquids to improve enantioselectivity.[3b,6] Ganske and Bornscheuer applied a mixture composed of an ionic liquid and 40% *tert*-butyl alcohol to synthesize a glucose fatty acid ester in good yield.[7] We also reported that 10% ionic liquid in a reaction system can enhance the rate and yield of nucleoside acylation.[8] While ionic liquids were recognized as promising solvents or additives in enzymatic reactions, however, Hinckley *et al.* found that the enzyme activity of laccase C from *Trametes sp.* decreased dramatically from half to less than 10% with an increase of concentration of 4-methyl-*N*-butylpyridium tetrafluoroborate from 10 to 20%.[9] Rogers and co-workers reported that an ionic liquid inactivated cellulase from *Trichoderma reesei*.[10] Bornscheuer and our group also noticed that CAL-B exhibited almost no activity in media composed of pure or too much ionic liquid.[7,8] Moreover, ionic liquids are relatively expensive, difficult to purify and not easy to obtain. The need for establishing novel additives in enzymatic reactions is still a challenge.

In this paper, we investigated several types of imidazole derivatives as additives, since they have similar structure to the cationic part of $[C_nMIM]^+$ ionic liquids, but avoid the possible interference of anions on the enzymatic activity. The results showed that *N*-methylimidazole enhanced lipase activity markedly, even better than that the ionic liquid in the reaction of acylation of ribavirin with divinyl adipate catalyzed by CAL-B (Scheme 1). The novel additive is very promising in enzymatic synthesis and useful for understanding the behavior of ionic liquids in biocatalysis.

The starting point of the present work was to identify what structure of imidazole derivatives could promote the reaction. Therefore, four types of imidazole derivatives, including imidazole, *N*-methylimidazole, 2-methylimidazole and 4-methylimidazole, were investigated. 0.1 mmol ribavirin with 4 equiv. divinyl adipate and 5 mg CAL-B was added to 2 ml solvent containing 10% of imidazole derivative and 90% acetone. The reaction was incubated at 50 °C and shaken at 200 rpm. Samples were taken from the reaction mixture after fixed intervals of time and determined by HPLC. Elution was performed with a mixture of methanol–water (40/60, v/v) at 1 mL min^{-1}. The results are shown in Fig. 1. Compared with no additive in acetone, *N*-methylimidazole and imidazole promoted the reaction rate markedly, while 2-methylimidazole and 4-methylimidazole had no positive effect. The best results were obtained upon adding *N*-methylimidazole. The yield was more than 60% after 0.5 h and reaction reached equilibrium at 4 h with 96% yield, while the yield in acetone is only 25%.

Next, the influence of the substituent group on the nitrogen was investigated. The yield declined when the substituent was changed from methyl to a larger one as shown in Fig. 2. The benzyl and butyl groups have more steric hindrance which prevent the imidazole derivative from participating in the reaction, and even had a negative effect. Several control experiments were designed to demonstrate that the acylation of ribavirin with divinyl adipate is an enzyme catalyzed process. When the reactants were incubated with denatured CAL-B (pre-treated with urea at 100 °C for 6 h) or/and *N*-methylimidazole, no product was detected, ruling out the possibility that the polymeric support or the *N*-methylimidazole had promoted the process. Therefore, the role of *N*-methylimidazole in the system is enhancing the activity of lipase. A proposed mechanism is considered. *N*-methylimidazole, which is similar to the residue of histidine, may form a hydrogen bond with the hydroxyl group, due to the electron pair of nitrogen. It activates the hydroxyl group and makes the substrate more

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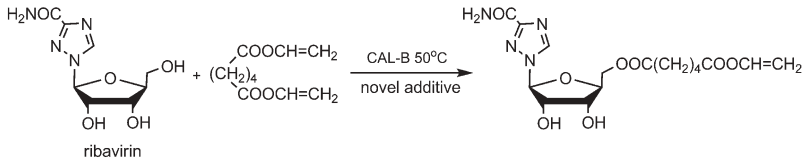

Scheme 1 The reaction of acylation of ribavirin with divinyl adipate catalyzed by CAL-B at 50 °C.

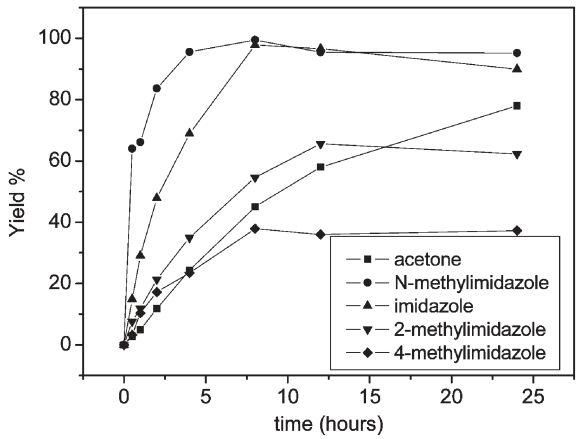

Fig. 1 Different imidazoles were added to the reaction of acylation of ribavirin with divinyl adipate catalyzed by CAL-B at 50 °C.

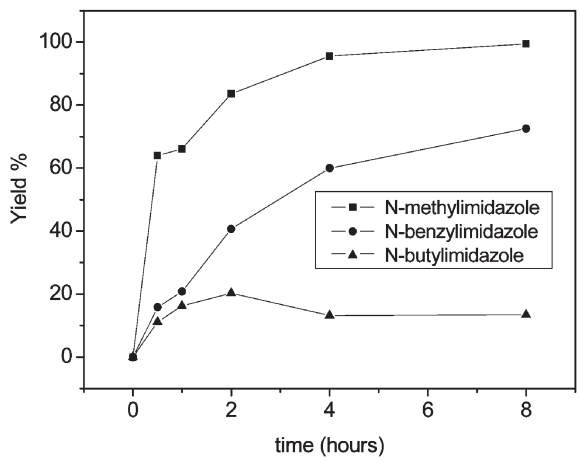

Fig. 2 The influence of the substituent group on imidazole on the reaction of acylation of ribavirin with divinyl adipate catalyzed by CAL-B at 50 °C.

nucleophilic. Compared with other imidazole derivatives, the methyl group on the nitrogen could make *N*-methylimidazole bind hydrogen more readily. However, the *N*-methylimidazole would also interact with the active site through hydrogen bonding, which leads to inhibit the reaction when the amount of *N*-methylimidazole is too high.

An interesting result was observed during further optimization of *N*-methylimidazole dosage shown in Fig. 3. The reaction was disfavored when too much *N*-methylimidazole was added. The activity of the lipase decreased sharply with an increase of *N*-methylimidazole in the medium from 10 to 40%. It was found that 10% is the best level in the reaction system, which is very similar to that of 1-butyl-3-methylimidazolium tetrafluoroborate

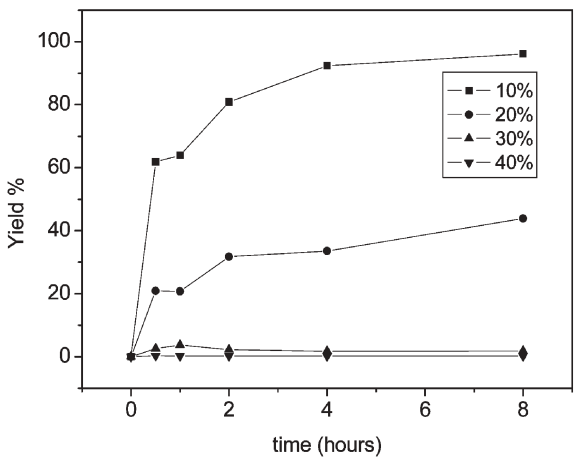

Fig. 3 Different percentages of *N*-methylimidazole were added to the reaction of acylation of ribavirin with divinyl adipate catalyzed by CAL-B at 50 °C.

([BMIM]BF_4).[8] The reaction no longer occurred when *N*-methylimidzaole was added at more than 40% in the system. The same results were obtained when the content of ionic liquid was increased up to the same percentage. It seems that *N*-methylimidazole has the same effect as [BMIM]BF_4 in the enzymatic reaction.

In order to prove that the *N*-methylimidazole has the same role as the cations of the ionic liquid, the influence of anion was investigated (Fig. 4). As seen from the figure, the reaction rate in the presence of added $NaBF_4$ or KPF_6 was much lower than that in acetone. This demonstrated that the *N*-methylimidazole improved the activity of lipase and is in agreement with the fact that the BF_4^- and PF_6^- destabilize the lipase.[11] Also, PF_6^- has

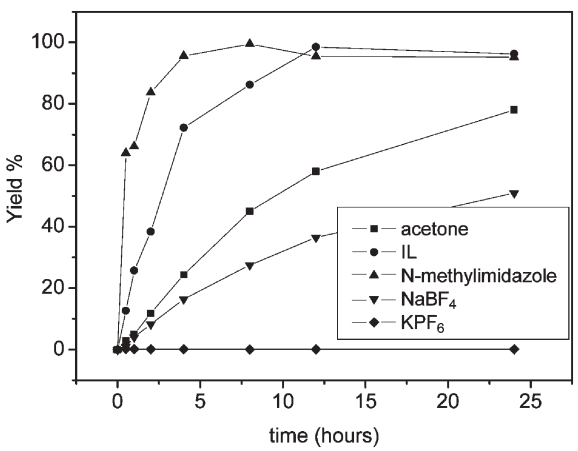

Fig. 4 The influence of anions in ionic liquids on the reaction of acylation of ribavirin with divinyl adipate catalyzed by CAL-B at 50 °C.

much more negative effect on the lipase, which is in agreement with our former experiment.[8] A much better result was obtained when the *N*-methylimidazole was used as additive free of BF_4^- compared with $[BMIM]BF_4$. It is assumed that the results obtained in $[BMIM]BF_4$ consisted of the positive and negative effect of the cation and anion parts, respectively.

In conclusion, the reaction rate was accelerated remarkably by adding 10% *N*-methylimidazole in the absence of anion. We discovered that *N*-methylimidazole activated the lipase similarly to $[C_nMIM]^+$. This method provides another possibility to investigate the role of ionic liquids in enzymatic reactions. Further study of the interaction mechanism between *N*-methylimidazole and enzyme is under progress.

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