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学位論文の概要及び要旨

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題　　目　Development of Lipase-catalyzed Reactions in an Ionic Liquid System

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The value of an enzymatic reaction in organic synthesis is greatly increased by its environmentally friendly aspect. Lipase PS from *Burkholderia* cepacia is one of the most widely used enzymes applicable for various substrates, however, the activity in nonaqueous media is reduced and it has been reported that the enantioselectivity is significantly dependent on the solvent system. Therefore, development of a strategy to improve lipase reaction performance in an organic solvent system is desirable.

Room temperature ionic liquids are a new class of solvents and have attracted growing interest recently because of their unique physical and chemical properties. Bioscience and Technology established that lipase-catalyzed transesterification could be conducted in an ionic liquid solvent system; the enantioselectivity was dependent on the anionic part of the imidazolium salts.

On the activation of lipase, several methods have been reported in a nonaqueous medium, for example, lipid coating mediated activation, salt-mediated activation etc. Based on these results, we investigated the possibility of regulating the enantioselectivity and reactivity of lipase PS and CRL using i-Pr_2O as solvent. We expected that optimization of an appropriate combination of the anionic part and the imidazolium cation of the ionic liquids might make it possible to design a useful regulator of lipase-catalyzed reaction.

1.　Additive effect of　on the lipase-catalyzed transesterification

To enhance enzyme enantioselectivity in a nonaqueous solvent system, the simplest is a addition of a proper compound that affects the enzyme activity, we therefore investigated lipase-catalyzed acylation of (±)-1-phenylethanol ((±)-1a) in the presence of various additives, in particular, ionic liquid additive using i-Pr_2O as solvent. Except for thiocrown ether, [bmim][BF_4], [bdmim][PF_6], two types of imidazolium salts, [bdmim][cetyl-PEG10-sulfate](IL1) and [bmim][cetyl-PEG10-sulfate] (IL2) were synthesized. There was a clear contrast in modification property between these two ionic liquids to the lipase PS-C-catalyzed reaction, and we discovered that ionic liquid IL1 worked as an excellent additive to enhance enantioselectivity, and high E value was recorded even　when IL1 was used as additive in the Celite free PS (CF-PS)-catalyzed reaction.

2.　Activation of lipase by the ionic liquid coating

From several recent reports about the acceleration effect of lipase, we hypothesized that the coating method might be the key point for activation of lipases by an ionic liquid; lyophilization of an enzyme in the presence of the ionic liquid might be essential to activate the lipase.

So IL1-coated-PS, Celite free lipase PS (CF-PS) and other ionic liquids coated PS were

prepared, the results of transesterification of (±)-1-phenylethanol using these ionic liquid-coated enzymes are tremendous. A remarkable acceleration was accomplished using IL1-PS while excellent enantioselectivity was maintained. These results seem to suggest that the present activation might be caused by the result of cooperation of polyoxyethylenealkyl group and a cationic part of the ionic liquid. It was confirmed that lyophilization of an enzyme in the presence of the ionic liquid was essential to activate the lipase, because no significant acceleration was recorded when CF-PS was mixed with IL1 melted by warming, and the resulting enzyme was used immediately for acylation.

Since it was anticipated that alkyl sulfate anion might have an impact on the lipase reactivity, we next attempted to evaluate polyoxyethylenealkyl sulfate anion using (±)3-Hydroxypentanenitrile ((±)-1b) as a model substrate. Ten types of ionic liquid coated lipase PS and Brij56-PS were prepared and used as catalysts for transesterification of (±)-1b. We discovered that IL1 was the best ionic liquid to activate lipase PS. The reaction rate was drastically accelerated and reached 98-fold acceleration over commercial lipase PS-C when IL1-supported lipase PS was used as catalyst.

We further discovered a very interesting activation property of IL1-PS: the rate of acceleration was significantly dependent on the substrates. A truly remarkable acceleration was accomplished for some compounds, even 1100 fold acceleration was obtained. and we demonstrated the effect of IL1 on the enantioselectvity was also dependent on the substrate.

Since it was anticipated that the supporting effect would be general for lipases, we next investigated the IL1-supporting effect of *Candida rugosa* lipase (CRL). As results, Both enantioselectivity and reaction rate were improved when the reaction was carried out using IL1-supported CRL as catalyst. Further, it was found that IL1 support significantly stabilized CRL in the organic solvent system, no drop in reactivity was observed when IL1-CRL-c was placed in dry hexane for a week at rt, while the reactivity was completely lost if commercial CRL was placed in hexane for the same period.

3. Recycling use IL1-PS

We attempted to demonstrate the recycling use of enzyme in the fluorine-substituted hydrophobic ionic liquid, 1-butyl-3-methylimidazolim 2,2,3,3,4,4,5,5-octafluoropentyl sulfate [bmim][C5F8] solvent system using 4-phenyl-3-butene-2-ol (1j) as substrate. To our delight, the desired product was obtained with excellent enantioselectivity using IL1-PS as catalyst, and five repetitions of this process showed no significant drop in the reaction rate.

4. Investigation of the origin of this IL1-mediated activation

In order to make sure that why such remarkable acceleration was accomplished by ionic liquid coating of the enzyme, the origin of this IL1-mediated activation was investigated. At first we measured the kinetic parameters of *Burkholderia* cepacia (PS-C)-catalyzed transesterification of 1b, and got the reaction parameters: Vmax, Kcat, Km Kcat/Km. then to confirm the binding of the ionic liquid with the lipase protein, MALDI-TOF Mass experiment were carried out.

5. Synthesis of optically active cycloalkenol via combination process of enzyme-catalyzed reaction and ring-closing metathesis(RCM) reaction

Based on the lipase-catalyzed acylation and olefin methathesis reaction that was a very effective means for preparing ring compounds, a novel and convenient synthesis method of optically active cyclic alkenol was described, with this combination process of lipase-catalyzed reaction and RCM reaction, a practical strategy for preparing optical ring alknol with excellent enatioselectivity succeeded.