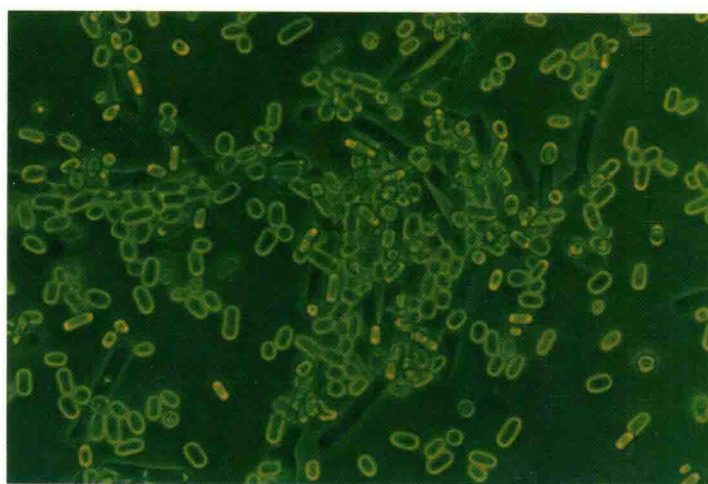


Future Directions in Biocatalysis

Editor: Tomoko Matsuda



Future Directions in Biocatalysis

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Future Directions in Biocatalysis

Preface

Important topics in biocatalysis for organic synthesis are described in this book, for experts and non-experts. Especially, the book focuses on those reactions that are under development now and will be more significant in the future. Therefore, each chapter describing a specific theme summarizes not only the present state but also the direction of the research. The prospects and dreams that will become possible, using biocatalysis, in the future to construct a sustainable society are also included.

The book consists of four sections: the enzymatic reaction under unusual conditions, unique biocatalytic reactions, valuable compounds synthesized using biocatalysis and latest molecular biology methods to make useful biocatalysts. The first section dealing with unusual reaction conditions for biocatalysis begins with the use of ionic liquid as a solvent to develop green chemistry. Then, the reaction under extreme temperatures, use of light energy as a driving force to proceed biocatalysis and catalysis by enzyme-metal combinations are described. The second section covers very unique reactions such as carboxylation using decarboxylases and carbon dioxide, Baeyer-Villiger reaction using monooxygenases, reactions in aldoxime-nitrile pathway including dehydration in aqueous solvent and addition of nitrile to carbonyl compounds to synthesize chiral cyanohydrin. The third section highlights novel compounds synthesized using biocatalysts. Chiral heteroatom-containing compounds such as chiral phosphorus compounds, polymer materials and sugars are selected to demonstrate the usefulness of biocatalysts. The last section describes the use of molecular biology technique to find novel biocatalysts. Alcohol dehydrogenase and decarboxylase are chosen as an example for the use of techniques since detailed and interesting researches have been conducted using these enzymes.

Through this book, I hope to introduce the novelty and future directions in biocatalysis. Importantly, I wish to contribute to conserve and beautify the natural environment by editing the book, showing the power of enzymes, treasure from Mother Nature, to catalyze the necessary reaction for mankind in an environmental-friendly manner.

Tomoko Matsuda

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Part One

Novel reaction conditions for biotransformation

Chapter 1

Biotransformation in ionic liquid

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Abstract

The use of ionic liquids (ILs) to replace organic or aqueous solvents in biocatalysis processes has recently gained much attention and great progress has been accomplished in this area; lipase-catalyzed reactions in an IL solvent system have now been established and several examples of biotransformation in this novel reaction medium have also been reported. Recent developments in the application of ILs as solvents in enzymatic reactions are reviewed.

1. INTRODUCTION

Ionic liquids (ILs) have very good properties as reaction medium in chemical reactions: they are non-volatile, non-flammable, have low toxicity and good solubility for many organic and inorganic materials.¹ It has long been recognized that an enzymatic reaction proceeds in an aqueous buffer solution under appropriate pH conditions and an enzyme quickly loses its activity in a highly concentrated aqueous salt solution.² Therefore it seems a foolish notion that enzymatic reaction occurs in a salt medium from the standpoint of biology. However, the use of ILs to replace traditional organic solvents in chemical reactions has recently gained much attention, and even as a novel reaction medium for biotransformation. Lipase-catalyzed reactions in an IL solvent system have now been established,^{1b–c} and several types of non-lipase enzymatic reactions have also been reported recently. I wish to review recent progress in the area of “enzymatic reactions in an IL solvent system” in this chapter.

2. IONIC LIQUIDS AS A REACTION MEDIUM FOR BIOTRANSFORMATION

Cull and co-authors^{3a} reported a microbe-mediated transformation of benzamide from benzonitrile in a mixed solvent of IL, 1-butyl-3-methylimidazolium

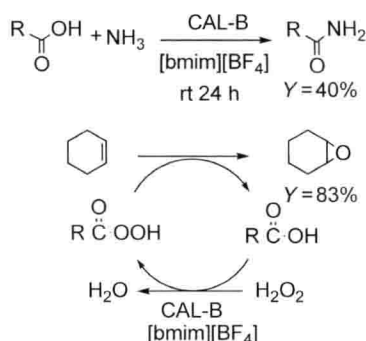


Figure 1: The first enzymatic reaction system conducted in a pure ionic liquid solvent system.

hexafluorophosphate ([bmim][PF₆]), with water (1:4) in July 2000. Then Russell and co-authors^{3b} reported that thermolysin-catalyzed amidation of CBz-asparagine with L-phenylalanine methyl ester proceeded in a mixed solvent of [bmim][PF₆] with aqueous buffer solution. These examples showed that the IL had no inhibitory action against the enzymes because [bmim][PF₆] was insoluble in water and enzymatic reactions took place in the water layer. The first example of enzymatic reaction in a pure IL solvent system was reported by the Sheldon group in December 2000.⁴ The authors successfully demonstrated two types of *Candida antarctica* lipase (CAL-B) catalyzed reaction in a pure IL: CAL-B catalyzed amidation of octanoic acid with ammonia and also the formation of octanoic peracid by the reaction of octanoic acid with hydrogen peroxide (Fig. 1).

However, the reactions were not enantioselective ones, though the most important aspect of the biocatalysis reaction should be in the enantioselective reaction. We^{5a} and Kragl⁶ independently reported the first enantioselective lipase-catalyzed reaction in February–March 2001. Since lipase was anchored by the IL solvent and remained in it after the extraction work-up of the product, we succeeded in demonstrating that recyclable use of the lipase in the [bmim][PF₆] solvent system was possible (Fig. 2).^{5a}

Typically the reaction was carried out as follows: to a mixture of lipase in the IL were added this racemic alcohol and vinyl acetate as the acyl donor. The resulting mixture was stirred at 35°C and the reaction course was monitored by GC analysis. After the reaction, ether was added to the reaction mixture to form a biphasic layer, and product acetate and unreacted alcohol were extracted with ether quantitatively. The enzyme remained in the IL phase as expected (Fig. 2). Two months later, Kim and co-workers^{7a} reported similar results and Lozano and Ibora^{7b,d} reported other examples of lipase-catalyzed reaction in June. Further Park and Kazlauskas^{7c} reported full details of lipase-catalyzed reaction in an IL solvent

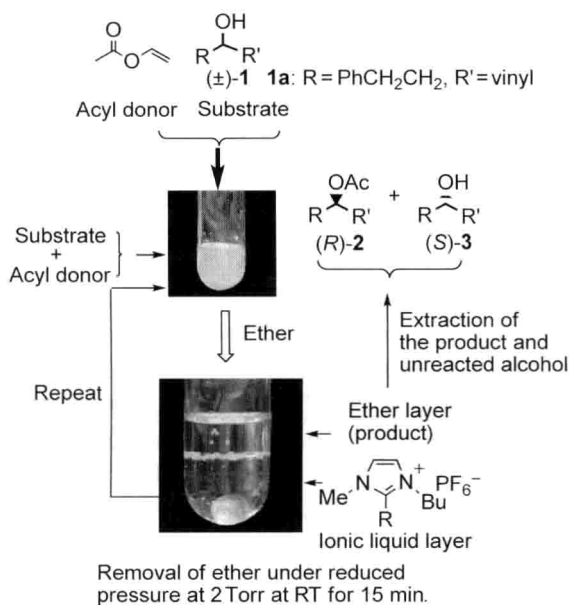


Figure 2: Lipase-catalyzed reaction system anchored to the solvent.

system in August 2001. Studies on the enzymatic reaction in an IL solvent system were thus launched in 2000–2001.

We initially tested *Candida antarctica* lipase using imidazolium salt as solvent because CAL was found to be the best enzyme to resolve our model substrate 5-phenyl-1-penten-3-ol (**1a**); the acylation rate was strongly dependent on the anionic part of the solvents. The best results were recorded when [bmim][BF₄] was employed as the solvent, and the reaction rate was nearly equal to that of the reference reaction in diisopropyl ether. The second choice of solvent was [bmim][PF₆]. On the contrary, a significant drop in the reaction rate was obtained when the reaction was carried out in TFA salt or OTf salt. From these results, we concluded that BF₄ salt and PF₆ salt were suitable solvents for the present lipase-catalyzed reaction.^{5a} Acylation of **1a** was accomplished by these four enzymes: *Candida antarctica* lipase, lipase QL from *Alcaligenes*, Lipase PS from *Burkholderia cepacia* and *Candida rugosa* lipase. In contrast, no reaction took place when PPL or PLE was used as catalyst in this solvent system. These results were established in March 2000 but we encountered a serious problem in that the results were significantly dependent on the lot of the ILs that we prepared ourselves. The problem was very serious because sometimes the reaction did not proceed at all. So we attempted to purify the ILs and established a very successful procedure (Fig. 3): the salt was first washed with a mixed solvent of hexane and ethyl acetate (2:1 or 4:1), treated with activated charcoal and passed into activated alumina neutral type I as an acetone solution. It was evaporated and dried under reduced

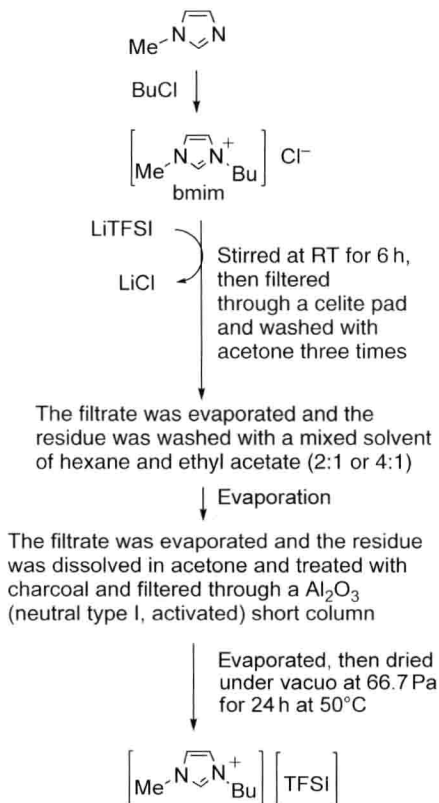


Figure 3: Purification protocol of imidazolium ionic liquid.

pressure at 50°C for 24 h to obtain very clean imidazolium salt. Using the ILs, we succeeded in obtaining more reproducible results; I recommend this as also being very useful to recycle the IL. In fact, we always recycle our ILs after the reaction and have not wasted any in the past. *We are still using ILs that have a 7-year history.* After establishing the reproducibility of our results of lipase-catalyzed reaction, we submitted our first paper in December 2000 and the paper was accepted on January 5, 2001. Although we lost the chance to be the first to publish in the field for this reason, we learned many things about ILs during that time and these are now important bases of our research group. Very pure ILs are commercially available now and we can use them, but I imagine that all research groups encountered the same problem in the early days of this field, because very clean ILs are required for a biocatalysis system compared to chemical reactions. This story highlights a very important point; we should pay attention to the quality of the IL when we evaluate the appropriate one for our desired biocatalyst reaction.