doi:10.1246/cl.130171
Published on the web May 15, 2013

Synergetic Activation of Lipase by an Amino Acid with Alkyl–PEG Sulfate Ionic Liquid

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The synergetic effect of amino acids and an ionic liquid, 1-butyl-2,3-dimethylimidazolium α-cetylpolyoxyethylene(10) ether sulfate (IL1), as coating materials on lipase was discovered: coating on lipase PS using a certain amino acid with IL1 was found to be very effective for accelerating the lipase-catalyzed transesterification of secondary alcohols while maintaining perfect enantioselectivity.

Lipases are among the most widely used enzymes applicable for various substrates; however, the reaction rate and enantioselectivity depend significantly on both the substrates and reaction media: the reaction rates are generally dependent on the reaction media, and very slow or poorly enantioselective reactions are sometimes obtained.[1]

We have been investigating enzymatic reactions in the ionic liquid (IL) solvent system and have established that various types of ILs are applicable as solvents for biochemical reactions.[2–4] We further developed a powerful method for the activation of lipase protein through coating with an ionic liquid: *Burkholderia cepacia* lipase coated with the ionic liquid, 1-butyl-2,3-dimethylimidazolium α-cetylpolyoxyethylene(10) ether sulfate (IL1),[5,6] (IL1-PS), displayed excellent reactivity for many substrates in conventional organic solvents[5] and ionic liquids.[7]

Luo and co-workers reported the preparation of chiral imidazolium salts derived from proline.[8] Inspired by their work, we prepared two types of chiral pyrrolidine-substituted imidazolium α-cetylpolyoxyethylene(10) ether sulfate, and found that (*R*)-3-butyl-2-methyl-1-(pyrrolidin-2-ylmethyl)-1*H*-imidazol-3-ium α-cetylpolyoxyethylene(10) ether sulfate (D-ProMe) derived from D-proline worked as an excellent activating agent for lipase PS (Figure 1).[9] An extraordinary acceleration was accomplished with perfect enantioselectivity for the D-ProMe-PS-catalyzed reaction, and a reaction 58 times faster (vs. lipase PS) was recorded.[9] However, more simple coating materials need to be developed for the activation of lipase. We herein report the synergetic activation of amino acids and IL1 as a coating material for lipase.

Amino acids have been used as stabilizers of enzymes during the purification process. For example, commercial lipase PS contains ca. 20 wt % glycine as an essential stabilizer during the preparation of the lipase protein through the lyophilization process.[5b] We investigated the role of glycine and established that it worked only as a stabilizer of the enzyme and had no influence on the reactivity of lipase PS.[5b] Since it was anticipated that a chiral amino acid may modify the enantioselectivity of an enzymatic reaction, we next prepared amino acid-coated lipase PS and investigated its properties in the transesterification of (±)-1-phenylethanol (**1a**) as a model substrate in the presence of vinyl acetate as an acyl donor in the *i*-Pr_2O

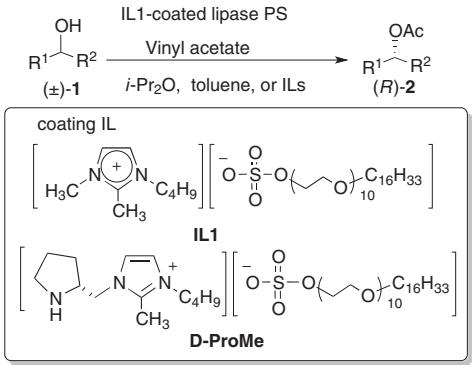

Figure 1. Ionic liquid-coated lipase PS-catalyzed reaction system.[5,9]

solvent system (Figure 2).[10] As shown in Figure 2, the coating of lipase PS with amino acids neither accelerated the reaction nor modified its enantioselectivity, although coating lipase with L-aspartic acid (Asp) and L-cysteine (Cys) caused a significant reduction thereof. To our delight, we discovered very interesting synergetic activation of an enzyme with an amino acid and IL1. A significant acceleration was obtained by coating lipase PS with a combination of the amino acid and IL1, which was prepared by treating glycine-free PS with 100 mol equiv of L-amino acid and IL1: 100- to 300-fold acceleration was found compared to the native or amino acid-coated lipase PS. It was found that the combination of IL1 and L-proline (Pro) was particularly effective in activating the lipase PS: 330-fold acceleration was accomplished by using L-proline and IL1-coated PS.

Since it was already established that glycine worked only as a stabilizer of the enzyme and had no influence on the reactivity of lipase PS, we next prepared amino acid-coated PS samples by using commercial lipase PS, which included 20 wt % of glycine, in the presence of IL1 just after removal of the supporting celite, and investigated their properties for the activation of lipase PS.

The coating effect between L-amino acids ($Rate^1$ in Figure 3) and D-amino acids ($Rate^2$ in Figure 3) was compared:[10] (±)-(*E*)-4-phenylbut-3-en-2-ol (**1b**)[5b] was used as a model substrate, because the reaction rate of lipase PS for this alcohol was not satisfactory, while the enantioselectivity was perfect. As shown in Figure 3, different activation effect levels were found for cysteine-, proline-, tyrosine-, and methionine-coated enzymes. Interestingly, coating with nonnatural D-amino acids generally led to slightly greater acceleration than coating with natural L-amino acids. However, the difference was not significant compared to that between D-ProMe and L-ProMe: D-ProMe gave rise to double the acceleration of L-ProMe.[9]

To gain further insight into the synergetic activation of lipase PS with amino acids and IL1, we conducted kinetic

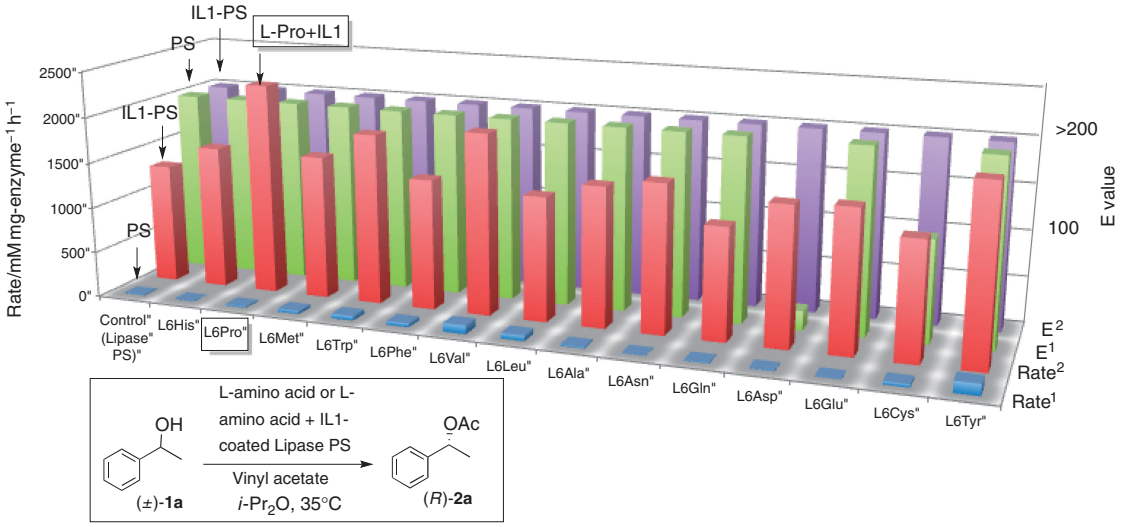

Figure 2. Effect of coating on lipase PS only with an amino acid (Rate^1 and E^1) or with both of an amino acid and IL1 (Rate^2 and E^2) on transesterification of (±)-1-phenylethanol (**1a**) using vinyl acetate as acyl donor. Enantioselectivity was evaluated by the E value.[11] Rate^2 of the control is the result of IL1-coated PS.

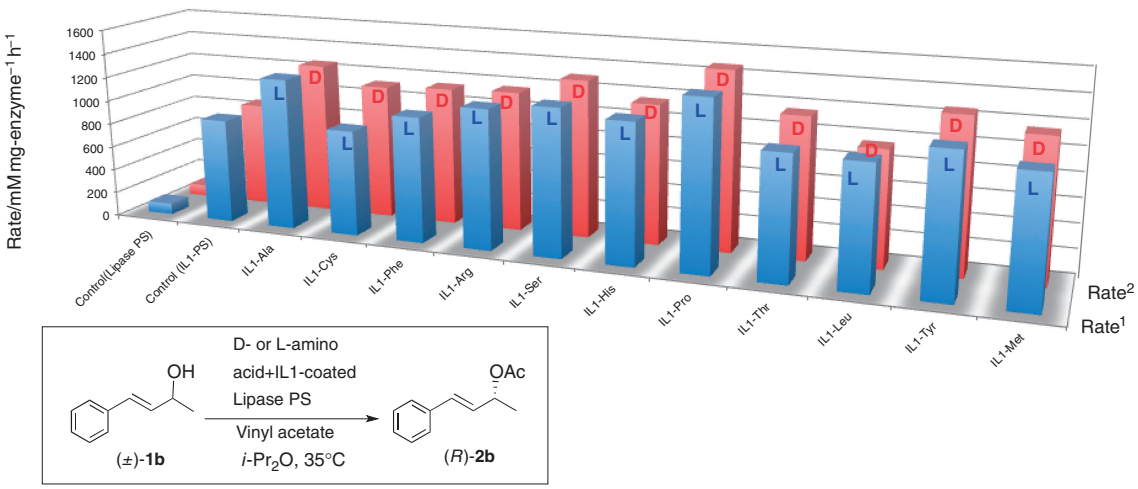

Figure 3. Effect of coating on lipase PS with L-amino acids or D-amino acids with IL1 on transesterification of (±)-4-phenylbut-3-en-2-ol (**1b**). Rate^1 = L-amino acid with IL1. Rate^2 = D-amino acid with IL1. Perfect enantioselectivity (E >200) was obtained for all reactions.

experiments on three types of IL1-coated lipase PS samples using (*R*)- and (*S*)-(*E*)-4-phenylbut-3-en-2-ol (**1b**) as substrates; the results are shown in Table 1. A significant increase in the K_{cat} value was observed for the (*R*)-isomer (37-fold acceleration was obtained for IL1 + D-Pro compared to the control), while only a small acceleration was obtained for the (*S*)-isomer (7-fold acceleration was recorded for IL1 + D-Pro compared to the control) (Table 1). On the other hand, the K_m values were increased slightly for both enantiomers compared to those of PS (none) (Table 1), though it was difficult to identify the origin of these small differences.

We reported earlier that the K_{cat} value of the IL1-coated lipase PS-catalyzed reactions was increased compared to those of native lipase PS.[5b] Modified K_m values were also observed between enantiomers of the substrate alcohol when lipase PS

Table 1. Results of transesterification of (*R*)- or (*S*)-4-phenylbut-3-en-2-ol (**1b**) using four types of enzymes

Substrate	Coating material	V_{max}[a]	K_m/M	K_{cat}/min^{-1}	K_{cat}/K_m
(*R*)-**1b**	None (control)	1.9×10^{-3}	1.3×10^{-1}	7.5×10^{-3}	5.9×10^{-2}
(*R*)-**1b**	IL1	8.7×10^{-2}	3.1×10^{-1}	3.5×10^{-2}	1.1
(*R*)-**1b**	IL1 + L-Pro	1.0×10^{-1}	3.5×10^{-1}	4.0×10^{-1}	1.1
(*R*)-**1b**	IL1 + D-Pro	7.0×10^{-2}	2.3×10^{-1}	2.8×10^{-1}	1.2
(*S*)-**1b**	None (control)	5.0×10^{-4}	1.4×10^{-1}	2.0×10^{-3}	1.5×10^{-2}
(*S*)-**1b**	IL1	5.8×10^{-2}	1.7	2.3×10^{-1}	1.3×10^{-1}
(*S*)-**1b**	IL1 + L-Pro	2.5×10^{-2}	4.3×10^{-1}	9.9×10^{-2}	2.3×10^{-1}
(*S*)-**1b**	IL1 + D-Pro	3.5×10^{-2}	9.2×10^{-1}	1.4×10^{-2}	1.5×10^{-1}

[a]M min^{-1} mg-enzyme^{-1}.

was coated with chiral imidazolium salts:[9] the K_m value of the (*S*)-isomer was reduced compared to that of PS when D-ProMe-PS was used as a catalyst, while the value was increased for the (*R*)-isomer (double that of native PS).[9] These results suggest that the cationic part of the ionic liquid and amino acid might bind with the lipase protein, causing a conformational change in the enzyme and contributing to the difference in K_m between the enantiomers. The chiral imidazolium cation might affect the enzyme reactivity strongly compared to amino acids when it binds with the protein. We also assume that the ionic liquid may bind with the enzyme protein and form an IL layer on the protein surface,[12] thus contributing to the increased flexibility of the enzyme protein. Since our ionic liquid has amphiphilic properties, this also contributes to the concentration of the hydrophobic substrate on the enzyme protein so that initial acceleration of the rate might be realized.

In conclusion, we have discovered a synergetic activation of the lipase protein through coating with a combination of certain amino acids and 1-butyl-2,3-dimethylimidazolium α-cetylpolyoxyethylene(10) ether sulfate (IL1). Although the induced activation is slightly less effective than that of the chiral pyrrolidine-substituted imidazolium salt (D-ProMe),[9] the present method is much more practical for attaining sufficient activation of lipase PS, because the coating material is just a mixture of an amino acid, such as L- or D-proline, with a simple imidazolium alkyl–PEG sulfate (Figure 3). Here, we suggest a possible way to realize the activation of a lipase by using a very simple methodology and hope that this may become an even more promising approach after refinement of the molecular design of the coating material. We believe that further investigation of the scope and limitation of this salt-mediated activation of enzymes will make it even more valuable.

The present work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 23360369), and also by a fund for Environmental Research from Tottori Prefecture. The authors are grateful to Dr. Yoshihiko Hirose of Amano Enzyme, Ltd., for providing the Celite-free lipase PS.

This paper is dedicated to Professor Teruaki Mukaiyama in celebration of the 40th anniversary of the Mukaiyama aldol reaction.

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