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Aqueous-Level Turnover Frequency of Lipase in Organic Solvent

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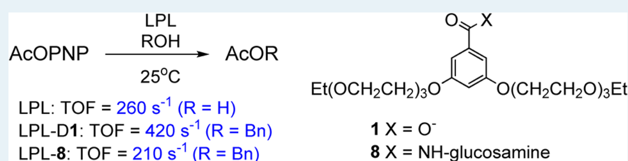
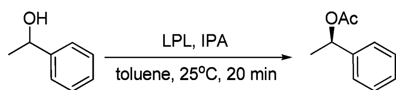
Supporting Information

ABSTRACT: In this work, we explored the activation of a lipoprotein lipase from *Burkholderia sp.* by surfactants, sugars, and sugar−surfactant combinations for enhancing its activity in organic solvent. The lipase was most strongly activated by the combination of dextrin and anionic surfactant **1**. As a result, its turnover frequency in anhydrous toluene reached 420 s^{-1}, which was comparable to its aqueous counterpart. It was also found that a glucosamine-headed surfactant **8** as a molecular mimic of the dextrin-**1** combination enhanced the turnover frequency of LPL to the aqueous level. As a rationale for such a high turnover frequency of lipoprotein lipase in anhydrous organic solvent, we speculate that the dextrin-**1** couple or its mimic **8** could provide an efficient water-mimicking hydrogen bonding network around the enzyme in addition to the contribution as the oil surrogate, thus leading to a large increase of active enzyme molecules.



KEYWORDS: *lipase, activation, surfactant, dextrin-surfactant combination, synergy effect*

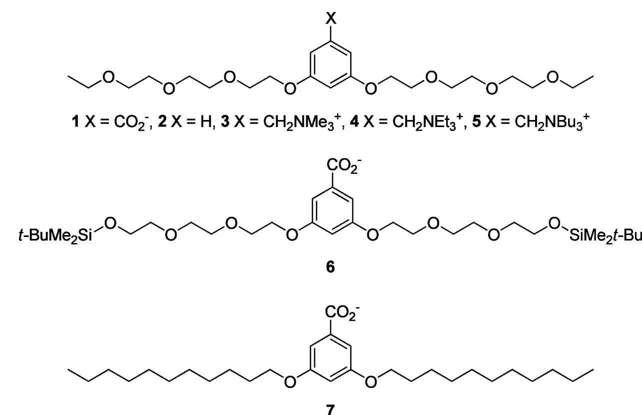
Table 1. Activities of LPL Preparations in the Transesterification of 1-Phenylethanol with Isopropenyl Acetate (IPA) in Toluene[a]



entry	additives[b]	level of conversion (%)
1	none	<1[c]
2	**1**	<3
3	dextrin and **1**	44[c,d]
4	dextrin	<1
5	dextrin and **2**	33
6	dextrin and **3**	17
7	dextrin and **4**	13
8	dextrin and **5**	10
9	dextrin and **6**	23
10	dextrin and **7**	11
11	sucrose and **1**	38
12	glucose and **1**	34
13	galactose and **1**	36

[a]The reactions for measuring the conversion % were performed with solutions containing lipase preparation (1.0 mg), 1-phenylethanol (0.50 M), and IPA (0.75 M) in toluene (0.2 mL) at 25 °C for 20 min. The reactions for measuring the initial rates were performed with solutions containing lipase preparation (1.0 or 10 mg), 1-phenylethanol (0.50 M), and IPA (0.75 M) in toluene (1 mL) at 25 °C. [b]Entry 1: LPL (100%); entries 2 and 4: LPL (25%), dextrin or **1** (75%); entries 3, 5−13: LPL (25%), surfactant (25%), and sugar (50%). [c]Initial rates: 1.0 (native LPL) and 1.5×10^3 mM h^{-1} mg^{-1} (LPL in the presence of dextrin and **1**). The initial rates were measured in duplicate. [d]The enantiomeric excesses of product and remaining substrate at 44% conversion were >99% and 79%, respectively, which gave E = >200.

Chart 1. Surfactants for the Activation of LPL



Enzymes are of great use as the catalysts for asymmetric transformations in organic synthesis.[1−3] Their broad use, however, is often hampered by their poor activities in organic solvents, which are employed as the media for most organic transformations. The enzymatic activity in organic solvent has been known to be several orders of magnitude lower than its aqueous counterpart. Accordingly, many efforts have been made for enhancing the activity of enzyme in organic solvent.[4] Some representative examples include the activations of proteases and lipases by inorganic salts,[5−7] surfactants,[8−10] and ionic liquids.[11−14] These approaches, however, still need further improvements for the practical applications of activated enzymes in organic solvent. Over the past decade, we have been developing the procedures using an enzyme and a metal catalyst

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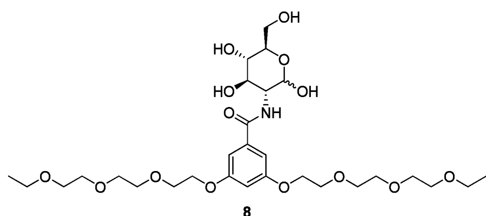
Table 2. Kinetic Parameters of LPL Preparations for the Hydrolysis and Transesterifications of *p*-Nitrophenyl Acetate (AcOPNP)[a]

AcOPNP + ROH —(LPL, buffer or toluene, 25°C)→ AcOR + HOPNP

entry	lipase	ROH	k_{cat} (s^{-1})	K_m[b] (M)	k_{cat}/K_m (s^{-1}/M)
1	LPL	HOH	2.6×10^2	4.5×10^{-4}	5.8×10^5
2	LPL-D1[c]	HOH	3.2×10^2	5.1×10^{-4}	6.3×10^5
3	LPL	$PhCH_2OH$	6.0×10^{-2}	2.4×10^{-1}	2.5×10^{-1}
4	LPL-D1[c]	$PhCH_2OH$	4.2×10^2	5.6×10^{-1}	7.5×10^2
5	LPL-D7[c]	$PhCH_2OH$	4.0×10^1	1.5×10^{-1}	2.7×10^2
6	LPL(**1**)[d]	HOH	5.3×10^2	1.3×10^{-3}	4.1×10^5
7	LPL-**8**[e]	$PhCH_2OH$	2.1×10^2	2.1×10^{-1}	1.0×10^3

[a]The V_{max} and K_m values were obtained from the Lineweaver−Burk plots of average rates per 1 mg of LPL (52% protein) measured in duplicate. Each k_{cat} was calculated using the equation $V_{max} = k_{cat}[E]_0$, where $[E]_0$ is total concentration of enzyme (MW 33 000). [b]For AcOPNP [c]The weight ratio between LPL, dextrin, and **1** or **7** = 1:2:1. The content of water determined by the Karl Fischer titration was less than 4% (w/w). [d]LPL in the presence of 1 mM **1**. [e]The weight ratio between LPL and **8** = 1:2.

Chart 2. Glucosamine-Headed Surfactant



in combination for the dynamic kinetic resolution (DKR) of alcohols, amines, and amino acids.[15−18] As a part of our continuous efforts in this field, we became interested in the strong activation of lipase for use in the DKR.[19,20] We herein wish to report that a dextrin−surfactant combination and its molecular mimic enhanced dramatically the activity of a lipase in organic solvent, and as a result, its turnover frequency (TOF) reached the aqueous level.

We chose a lipoprotein lipase (LPL) from *Burkholderia sp.* as a target enzyme for enhancing its activity in organic solvent. The commercial LPL (52% protein)[21] was poorly active when it was tested as the catalyst for the reaction of 1-phenylethanol with isopropenyl actetate (IPA) (entry 1, Table 1). We first tried to activate the LPL with an anionic surfactant **1** (Chart 1) which had been used for the activation of a crude lipase in our previous work.[19] The LPL was lyophilized in the presence of **1** for activation, but its activity was still low (entry 2). We then freeze-dried the LPL in the presence of both dextrin and **1**. To our surprise, the activity of LPL increased dramatically (entry 3). In this case, the activity enhancement based on the rate of reaction was 1500-fold at an optimum weight ratio (1:2:1) of LPL, dextrin (D), and **1**. We thus found a highly active lipase formulation (LPL-D**1**). Interestingly, such a dramatic activation disappeared completely in case LPL was lyophilized with dextrin only (entry 4).

Next, we tested additional surfactants **2**−**7**[22] in the presence of dextrin for the activation of LPL to see a relationship between the structure of surfactant and the enzymatic activity. The absence of an anionic carboxyl group at the head of surfactant reduced the enzymatic activity substantially (entry 5). Interestingly, the presence of a cationic ammonium group ($CH_2NR_3^+$) at the head position reduced the enzymatic activity further (entry 6). It is notable that the enzymatic activity decreased with increasing the hydrophobicity and bulkiness of the ammonium group (entries 6−8). On the other hand, the introduction of a bulky hydrophobic group such as *tert*-butyldimethylsilyl (TBDMS) at the end of each hydrophilic tail also led to a significant decrease in the enzymatic activity (entry 9). A further activity decrease was observed in case the hydrophilic tails were replaced by hydrophobic alkyl chains (entry 10). We also examined the effects of different sugars as the partners of **1** on the enzyme activity. Mono- and disaccharides activated the enzyme equally well but less strongly than dextrin (entries 11−13). All of these results indicate that the combination of dextrin and **1** is the best among those tested for the activation of LPL. Here we speculate that the activity enhancements of LPL by the sugar-surfactant combinations may be the results of the dispersion of enzyme particles into the sugar−surfactant matrix with an increase of active enzyme molecules (probably enzymes with open lid). The dextrin-**1** combination appears to provide the best matrix for this job. In addition, it was found that LPL was stable and recyclable in the presence of dextrin and **1**. LPL-D**1** retained most of its original activity after it had been reused three times.

We determined the kinetic parameters (k_{cat}, K_m, and k_{cat}/K_m) of LPL-D**1** in water and organic solvent to compare those of its native counterpart. The kinetic parameters in water was determined for the hydrolysis of AcOPNP in buffer and those in organic solvent for the transesterification of AcOPNP with benzyl alcohol in toluene. The k_{cat}/K_m data from Table 2 clearly indicate that LPL-D**1** is as active as its native counterpart in water (compare entries 1 and 2) but 3 orders of magnitude more active than the latter in organic solvent (compare the entries 3 and 4). The dramatically enhanced activity of LPL-D**1** relative to its native counterpart in organic solvent was largely the result of an increase (7000-fold) in the turnover frequency (TOF, k_{cat}). Furthermore, the TOF of LPL-D**1** in organic solvent was as good as its aqueous counterpart. This implies that the activity of LPL under the saturated condition in organic solvent reached the aqueous level.

It is noteworthy that the TOF of LPL-D**1** is 1 order of magnitude larger than that of LPL-D**7**, which in turn is ca. 700-fold larger than that of its native counterpart (compare the entries 3−5). This observation suggests that the hydrophilic tails of **1** plays a crucial role in inducing the strong activation of lipase. We speculate that they could act as hydrogen acceptors for providing an efficient water-mimicking hydrogen bonding

network together with dextrin around the lipase in addition to their contribution as the oil surrogate (like the hydrophobic tails of 7), thus inducing the conformational change of lipase to the highly active form. We determined the kinetic parameters of LPL in the presence of a significant amount (1 mM) of **1** in water to see if **1** interacts with lipase as the oil surrogate in water. It was found that the TOF of LPL was increased by 2-fold with a 3-fold increase in the K_m (compare the entries 1 and 6). These results support that **1** could act as the oil surrogate to induce the conformational change of lipase for the activation although the diffusion of substrate into the active site may be inhibited.[23]

Finally, we tested **8**[22] as a simple molecular mimic of dextrin-**1** combination (Chart 2) to see if it can also activate the LPL equally well. We found that LPL-**8** was as active as LPL-D**1** in organic solvent (compare the entries 1 and 7). It is noted that **8** is structurally unique and different from the amphiphiles with a hydrophilic head and hydrobobic tails previously used for the activation of lipases.[8−14]

The TOFs of enzymes in water have been known to be usually in the range of $10-10^3$ s^{-1}.[24] As described in Table 2, the TOF of LPL in water and those of LPL-D**1** and LPL-**8** in toluene are also in this range. This implies that LPL provides a good example for the enzymes with similar TOFs in water and organic solvent. The K_m values of the activated LPL preparations in organic solvent, however, were orders of magnitude larger than their aqueous counterpart.[25] Accordingly, their overall catalytic efficiencies (k_{cat}/K_m) in organic solvent are still below the aqueous level.

In conclusion, we have demonstrated for the first time that a synthetically useful lipase activated by the dextrin-**1** couple or its mimic **8** displayed an aqueous-level TOF in organic solvent.[26] We believe that the highly active lipase preparations can be utilized as the catalysts for the fast kinetic and dynamic kinetic resolution. Further studies for these synthetic applications are in progress.

■ ASSOCIATED CONTENT

Supporting Information

Procedures for the syntheses of surfactants **1**−**8** with their ^1H and ^{13}C NMR spectra and for the kinetic measurements and analyses of native and treated lipases. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(22) The procedures for their syntheses are provided in the Supporting Information.

(23) Recently, it was reported that TOF of *Pseudomonas cepacia* lipase enclosed in the cholesterol-containing polysaccharide nanogels was significantly larger than that of its native counterpart for the hydrolysis of *p*-nitrophenylpropionate, suggesting that the cholesteryl group as the oil surrogate induced a preferred conformational change of lipase Sawada, S.-I.; Akiyoshi, K. *Macromol. Biosci.* **2010**, *10*, 353−358.

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(25) Several factors can contribute to the large K_m values of LPL preparations in organic solvent. They include the mass transfer barrier of insoluble enzyme preparations, the higher stability of substrate in organic solvent, and the inefficient binding of substrate by the interference of dextrin and surfactant present in or around the active site of enzyme.

(26) It is expected that the TOF value of LPL in organic solvent could be further enhanced if more efficient hydrogen bonding network is provided around the enzyme with the better dispersion of active enzyme molecules.