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The Structural Effect of Benzoate Surfactant Tails on the Activity of Lipoprotein Lipase in Organic Solvent

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Lipases are useful as catalysts for the synthesis of optically active compounds.[1–3] In particular, they are increasingly being used in asymmetric synthetic transformations to produce enantiopure pharmaceuticals.[4] Lipases show high activity in water, particularly at the water–oil interface. However, most organic compounds are insoluble in aqueous medium. Furthermore, they are often degraded or converted to side products by water during the reaction. Product recovery is also difficult from water, and the thermodynamic equilibria of many reactions are unfavorable in aqueous medium.[5] These problems can be overcome by using organic solvents as reaction media. Synthetic applications of lipases in organic solvents, however, suffer often from their poor activities in nonaqueous media. It has been known that the activity of native lipase in organic media is several orders of magnitude lower than its aqueous counterpart. Thus, various methods have been developed to enhance the activity of lipase in organic solvent. One of the most widely used methods is the use of surfactants. The Schiavon group reported on the improvement of enzymatic activity by poly(ethylene glycol)-type surfactants in 1985.[6] The Mine group reported that water-soluble nonionic Gemini-type surfactants enhanced the activities of commercially available lipases for the transesterification of alcohols.[7–9] Recently, the Kim group reported that benzoate-based surfactants (1 and 2, Figure 1) enhanced significantly the activity of lipoprotein lipase from *Burkholderia* species (BSLPL) in organic solvent.[10,11] Interestingly, surfactant 2 with two hydrophilic tails was much stronger activator than 1 with two hydrophobic tails. Lately, I reported that benzoate surfactants 3 with a hydrophobic ring at the end of each hydrophilic tail were even more effective than **2** in enhancing the activity of BSLPL.[12] Furthermore, **3b** was the best among them. These results encouraged me to explore the structural effects of surfactant tails on the activity of BSLPL. Now I wish to report that surfactants with a hydrophilic and a hydrophobic tail are superior to those with only hydrophilic or hydrophobic tails in the BSLPL-activating ability.

Four different surfactants **1**–**4** were chosen to explore the structural effects of surfactant tails. Surfactants **1**–**3** were prepared according to the literature procedures.[10–12] The synthesis of new surfactant **4** is described in Scheme 1. Methyl 3,5-dihydroxybenzoate was coupled with undecyl bromide to obtain **5**, which was then reacted with the bromo derivative of triethyleneglycol ethyl ether to afford **6**. Methyl ester **6** was hydrolyzed and then converted to the target **4** as the potassium salt. This synthesis was straightforward and provided high yields in most steps.

Surfactant-coated BSLPLs containing each benzoate surfactant were prepared according to the protocol reported previously.[13] The activities of native and surfactant-coated BSLPLs were measured as the specific activities in the acetylation of 1-phenylethanol in toluene at 25 °C. The results are described in Table 1.

Native BSLPL showed very low activity in toluene (entry 1). The activity of BSLPL was enhanced by two orders of magnitude if it was co-lyophilized with surfactant **1** with two long alkyl chains as the tails (entry 2). Surfactant **2** with oligoethylene glycol chains activated BSPL one order of magnitude more strongly (entry 3). This implies that benzoate surfactants with hydrophilic tails are more powerful activators than those with hydrophobic tails. Interestingly, surfactants 3a-b with an aromatic ring at the end of each oligoethylene glycol chain were even better than **2** in activating BSLPL (entries 4–5). This observation suggests that the presence of both hydrophilic and hydrophobic parts in each tail is beneficial to the activation pf BSLPL. And it is noteworthy that surfactant **3b** induced the strongest activation (entry 5). Finally, surfactant 4 also induced the similarly strong activation of BSLPL (entry 6). Thus, the comparison between three surfactants (1, 2, and 4) in lipase–activation power reveals that surfactants with both hydrophilic and hydrophobic tails are more powerful activators than those with only hydrophobic or hydrophilic tails.

The results from the recent molecular dynamics simulation study on the mechanism of lipase activation[14] suggest that the hydrophilic parts of surfactants contribute to the stabilization of enzyme by interacting with the hydrophilic surface of enzyme. On the other hand, the hydrophobic parts of surfactants have favorable interactions with hydrophobic residues around the active site of enzyme, thus helping the enzyme maintain active open conformation in organic solvent. Consequently, these suggestions provide theoretical rationale for insight into the relationship between surfactant structure and lipase activity.

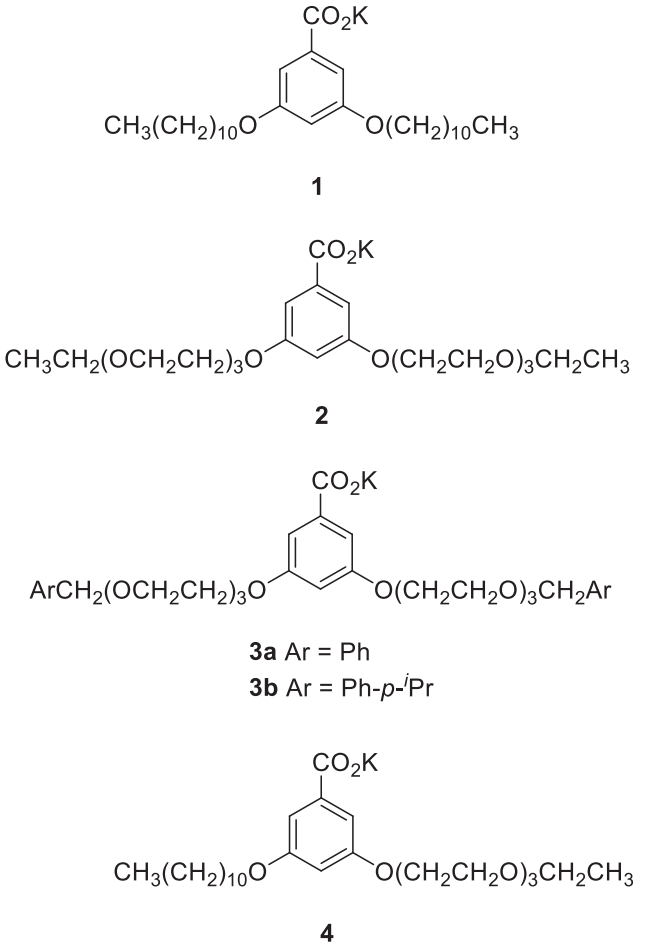
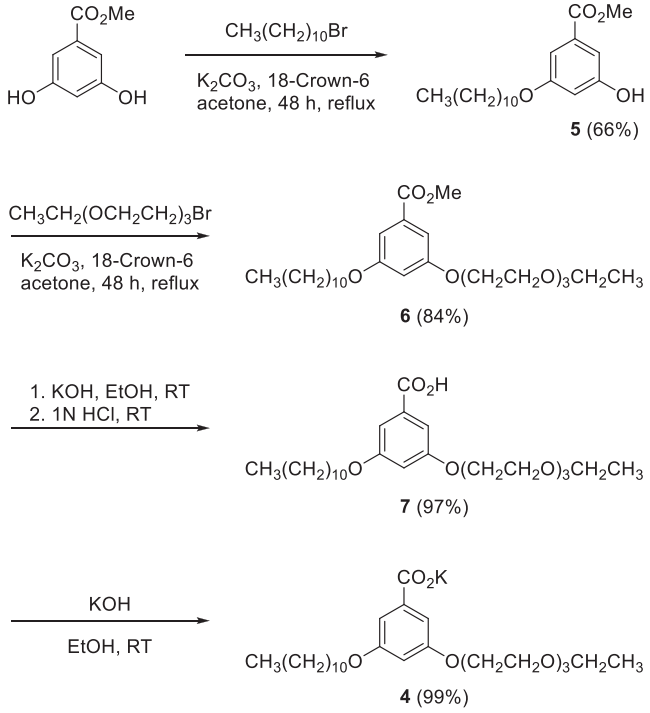


Figure 1 Structures of benzoate surfactants.



Scheme 1. Synthesis of benzoate surfactant **4**

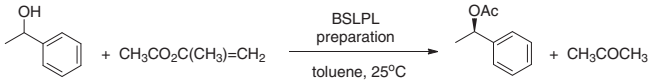
Surfactant **1** with no hydrophilic tails should have favorably strong interactions with hydrophobic parts around the active site of enzyme but poor interactions with hydrophilic parts of enzyme. To the contrary, surfactant **2** have two hydrophilic tails, thus interacting more favorably with hydrophilic parts of enzyme. Surfactant **2** also could have interactions with hydrophobic parts of enzyme owing to some hydrophobic nature of two tails although the hydrophobic interactions are relatively weaker than their hydrophilic counterparts. These more favorable interactions between surfactant **2** and enzyme should enhance enzymatic activity. Thus, BSLPL-2 (containing surfactant **2**) becomes more active than BSLPL-1. Surfactant **4** has both hydrophilic and hydrophobic tails, which allows for favorable interactions with both hydrophilic and hydrophobic parts of enzyme. As a result, BSLPL-4 displays higher activity compared to BSLPL-2. On the other hand, surfactant **3** has both hydrophilic and hydrophobic parts in each tail for favorable interactions with enzyme. These interactions make BSLPL-3, particularly BSLPL-3b, as active as BSLPL-4. It is concluded that surfactants with both hydrophilic and hydrophobic tails are superior to those with only hydrophilic or hydrophobic tails in the BSLPL-activating ability.

In summary, I prepared a new benzoate surfactant **4** and compared it with other surfactants **1**–**3** to see the structure–activity relationship in the activation of BSLPL. The results suggest that the BSLPL-activating ability of surfactant depends significantly on the hydrophilic and hydrophobic nature of its tails. Furthermore, *the tails of surfactant should have a balance in hydrophilic and hydrophobic nature for inducing high lipase activity in organic solvent*. It is believed that the structural characteristics of benzoate surfactants described in the manuscript can be used as the guidelines for the design of more efficient surfactants, leading to the preparation of highly active enzymes for use in organic solvent.

Experimental

General. All the commercially available starting materials and reagents were purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA), Acros Organics (Morris, NJ, USA), and TCI Co., LTD (Tokyo, Japan) and used without additional purification. Lipase from *Burkholderia* species was purchased from Amano Enzyme Inc. (Yokohama, Japan). All solvents were purified by purifying system; Ultimate Solvent System, Glass Contour from Nikko Hansen & Co., Ltd. (Osaka, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution at ambient temperature on a 300 MHz Fourier transform nuclear magnetic resonance (FT-NMR) Spectroscopy; AVANCE 300, Bruker (Billerica, MA, USA). Chemical shift are given in δ (ppm) relative to tetramethylsilane (TMS) as internal standard. Multiplicities were recorded as s (singlet), d (doublet), t (triplet), and m (multiplet). Reaction conversion was determined by HPLC; Agilent 1100 with (R, R)-Whelk-01 column. Molecular

Table 1. Activities of BSLPL preparations in the acetylation of 1-phenylethanol in organic solvent.[a]



Entry	Surfactant	BSLPL preparation	Specific activity (U)[b]	Enhancement
1	none	Native BSLPL	0.38	1
2	**1**	BSLPL-**1**	200	5.3×10^2
3	**2**	BSLPL-**2**	1500	3.9×10^3
4	**3a**	BSLPL-**3a**	1900	5.0×10^3
5	**3b**	BSLPL-**3b**	3000	7.9×10^3
6	**4**	BSLPL-**4**	2800	7.4×10^3

[a] BSLPL preparations containing BSLPL (25%, w/w), dextrin (50%, w/w), and surfactant (25%, w/w) were lyophilized in 1:1 (v/v) water-1,4-dioxane. The reactions were performed with solutions containing 1-phenylethanol (0.5 M), BSLPL preparation (1 mg), and isopropenyl acetate (1.5 equiv) in toluene at 25 °C. Rate measurements were done three times for each enzyme preparation. [b] 1 U = 1 μmole of product per hour per mg of BSLPL. The error range of the specific activity values is ±3% for all entries.

mass was measured by liquid chromatography–mass spectrometry (LC–MS); JMS-T100 L (AccuTOF), Jeol (Tokyo, Japan). All enzyme preparations were lyophilized by freeze dryer; FD-1000 or FDU-1200, Eyela (Tokyo, Japan).

Synthesis of 4. To the solution of methyl 3, 5-dihydroxybenzoate (810 mg, 4.81 mmol), K_2CO_3 (2.0 equiv) and 18-crown-6 (0.05 equiv) in acetone (20 mL) was added 1-bromoundecane (1.0 equiv) and the mixture was stirred under reflux for 48 h. After cooled down to r.t., the mixture was filtrated through celite-pad to remove insoluble materials. The filtrate was evaporated under reduced pressure and the residue was purified by silica gel chromatography to give **5** (1.02 g, 66% yield): ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.80 (t, J = 5.70 Hz, 3H), 1.19 (s, 14H), 1.35 (m, 2H), 1.68 (t, J = 6.70 Hz, 2H), 3.82 (s, 3H), 3.85–3.89 (m, 2H), 5.88 (s, 1H), 6.55 (s, 1H), 7.08 (d, J = 10.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 14.1, 22.6, 25.9, 29.1, 29.3, 29.3, 29.5, 29.6, 31.9, 52.3, 68.3, 107.1, 107.7, 109.0, 131.8, 156.8, 160.4, 167.2.

To the solution of **5** (1.0 g, 3.10 mmol), K_2CO_3 (2.0 equiv) and 18-crown-6 (0.05 equiv) in acetone (20 mL) was added 1-bromo-2-(2-(2-ethoxyethoxy)ethoxy)ethane (1.0 equiv) and the mixture was stirred under reflux for 48 h. After cooled down to r.t., the mixture was filtrated through celite-pad to remove insoluble materials. The filtrate was evaporated under reduced pressure and the residue was purified by silica gel chromatography to give **6** (1.26 g, 84% yield): ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.88 (t, J = 5.30 Hz, 3H), 1.20 (t, J = 7.01 Hz, 3H), 1.27 (s, 14H), 1.44 (m, 2H), 1.76 (m, 2H), 3.51–3.67 (m, 10H), 3.85 (m, 2H), 3.87 (s, 3H), 3.95 (t, J = 6.20 Hz, 2H), 4.14 (t, J = 3.81 Hz, 2H), 6.66 (s, 1H), 7.17 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 14.0, 15.1, 22.6, 25.9, 29.1, 29.3, 29.3, 29.5, 29.5, 31.8, 52.1, 66.9, 67.7, 68.3, 69.5, 69.8, 70.5, 70.6, 70.7, 70.8, 106.7, 107.5, 108.0, 131.8, 159.7, 160.1, 166.8.

To the solution of **6** (1.0 mmol) in ethanol (1.0 mL) were added aq. KOH (2.0 equiv). After stirring for 3 h at r.t., the reaction mixture was neutralized by 1 N HCl and concentrated under reduced pressure. The residue was extracted with methylene chloride and the solvent was evaporated to obtain **7** (1.41 g, 97% yield): ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.88 (t, J = 6.60 Hz, 3H), 1.21 (t, J = 6.99 Hz, 3H), 1.27 (s, 14H), 1.42 (t, J = 7.71 Hz, 2H), 1.78 (t, J = 7.09 Hz, 2H), 3.52–3.78 (m, 10H), 3.88 (m, 2H), 3.97 (m, 2H), 4.16 (t, J = 4.05 Hz, 2H), 6.71 (s, 1H), 7.23 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 14.1, 15.0, 22.6, 25.9, 29.1, 29.3, 29.3, 29.5, 29.6, 31.9, 66.6, 67.6, 68.3, 69.5, 69.7, 70.5, 70.6, 70.7, 107.2, 107.9, 108.5, 131.2, 159.7, 160.1170.9. TOF-MS (ESI+) calcd. For $[C_{26}H_{44}O_7 + Na]^+$ 491.29847, found 491.29687.

To the solution of **7** (0.5 mmol) in tetrahydrofuran (THF) (0.5 mL) was added KOH (0.1 M, 2.3 mL). After stirring for 1 h at r.t., the reaction mixture was concentrated under reduced pressure and further dried *in vacuo* for 24 h to afford **4** quantitatively. (252 mg, 99% yield): ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.76 (t, J = 6.62 Hz, 3H), 1.15 (t, J = 7.01 Hz, 3H), 1.22 (s, 14H), 1.38 (t, J = 7.68 Hz, 2H), 1.71 (t, J = 7.08 Hz, 2H), 3.43–3.75 (m, 10H), 3.81 (m, 2H), 3.93 (m, 2H), 4.10 (t, J = 4.09 Hz, 2H), 6.68 (s, 1H), 7.15 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 14.2, 14.8, 22.8, 25.4, 28.9, 29.3, 29.3, 29.6, 29.7, 32.0, 66.9, 67.5, 68.3, 69.4, 70.0, 70.6, 70.7, 70.9, 107.3, 107.9, 108.7, 131.5, 159.7, 160.2, 171.0.

Preparation of Surfactant-coated BSLPL Preparation. The enzyme powder (50 mg) was dissolved in deionized water (15 mL) and then mixed with dextrin and benzoate surfactant dissolved in 1,4-dioxane (15 mL) (enzyme: dextrin: surfactant = 1: 2: 1 weight ratio). The resulting solution was lyophilized to yield surfactant-coated BSLPL preparation.

Activity Measurements of BSLPL Preparations. Isopeopenyl acetate (1.5 equiv.) was added to a 20 mL vial containing BSLPL preparation (1 mg), 1-phenylethanol (0.5 mmol), and anhydrous toluene (0.5 M). The resulting solution was then shaken at 250 rpm at 25 °C. An aliquot was sampled periodically, filtered through a short silica pad, and analyzed by HPLC to determine the conversion % against time. The specific activity values were calculated from a slope of conversion %-time plot. The rate measurement for each BSLPL preparation was performed three times.

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