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A method to greatly improve the enantioselectivity of lipase-catalyzed hydrolysis using sodium dodecyl sulfate (SDS) as an additive

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Abstract—The addition of sodium dodecyl sulfate (SDS) resulted in a dramatic improvement of the enantioselectivity of the lipase-catalyzed hydrolysis of racemic butyl 2-(4-substituted phenoxy)propanoates, racemic butyl 2-(4-isobutylphenyl)propanoate, and racemic butyl 2-(6-methoxy-2-naphthyl)propanoate in an aqueous buffer solution. An increase in the *E* value by up to two orders of magnitude was observed for some esters. As to the effects of SDS on the structure of a lipase, FT-IR and fluorescence measurements suggest some conformational change and/or an increase of the flexibility of the lipase, although the native secondary structure of the lipase is held even in the presence of 100 mM SDS. The origin of the enantioselectivity enhancement brought about by the addition of SDS is briefly discussed on the basis of the values of the initial rates obtained for each enantiomer of the substrate.

1. Introduction

Lipase-catalyzed transformations have been established as an important tool in organic synthesis, because lipases display broad substrate specificity, yet retain relative high enantioselectivity not only in aqueous solution but also in organic media.[1] For this reason, organic chemists routinely use lipases as enantioselective catalysts in kinetic resolutions of racemates to provide easy access to enantiomerically pure compounds. These enzymatic reactions, however, do not always offer satisfactory results with high enantioselectivity toward non-natural substrates, such as pharmaceuticals, agrochemicals, or other synthetic targets. The structures of these substrates are widely different from those of the triacylglycerols of natural substrates for native lipases, that is, in other words, the acceptance of such non-natural substrates should force these enzymes to alter their active site conformations. Furthermore, the rigid enzymes seem to have difficultly in adjusting their conformational states for favorable interactions with substrates having undesirable structures.

A traditional approach for this problem is to screen enzymes from other sources in the hope of finding a more enantioselective one, although, this empirical method can seem tedious to organic chemists. Herein, we used sodium dodecyl sulfate (SDS), a denaturant, as an additive in the lipase-catalyzed hydrolysis of the racemic esters **1**–**9**. The addition of small amounts of SDS to the reaction medium was anticipated to cause a large flexibility in the lipase, yet retaining its function. This strategy is based on our findings that the enantioselectivity of lipase-catalyzed esterification was significantly improved by addition of aqueous SDS directly to suspended enzyme preparations in organic media.[2] It is certainly of value to see if this approach is valid even in lipase-catalyzed hydrolysis in an aqueous buffer solution.

2. Results and discussion

2.1. Effects of SDS on the enantioselectivity of lipase-catalyzed hydrolysis

Lipase MY from *Candida rugosa* catalyzes the enantioselective hydrolysis of racemic butyl 2-(4-substituted

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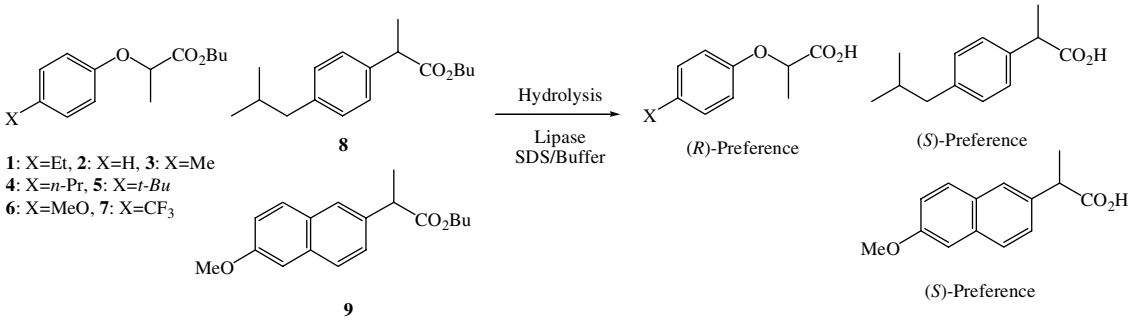

Scheme 1. Lipase-catalyzed hydrolysis of butyl 2-(4-substituted phenoxy)propanoates **1**–**7**, butyl 2-(4-isobutylphenyl)propanoate **8**, and butyl 2-(6-methoxy-2-naphthyl)propanoate **9**.

phenoxy)propanoates **1**–**7**, racemic butyl 2-(4-isobutylphenyl)propanoate **8** (butyl ester of ibuprofen) and racemic butyl 2-(6-methoxy-2-naphthyl)propanoate **9** (butyl ester of naproxen) in an aqueous buffer solution (Scheme 1). Lipase MY favors the (*R*)-enantiomers of **1**–**7** and the (*S*)-enantiomers of **8** and **9**, however these enantiomers of **1**–**9** have similar shapes, because of changes in priorities of the substituents. Some products of the hydrolysis of **1**–**7**, (*R*)-2-aryloxypropanioc acids, are well known herbicides and have other biological activities.[3] (*S*)-Naproxen and (*S*)-ibuprofen are also an important class of non-steroidal anti-inflammatory drugs.[4] Unfortunately, the enantioselectivity observed is low to moderate for these esters in an aqueous buffer solution without any additive (Table 1), the *E* value of which would not be sufficient for an effective resolution. To improve the enantioselectivity, various concentrations of SDS were tested as an additive in the lipase-catalyzed hydrolysis using **1** and **9** (Fig. 1). As can be seen in Figure 1, the addition of SDS significantly increases the enantioselectivity toward both substrates. The optimum concentration of SDS (100 mM) for **1**, however, is appreciably different from that (1 mM) for **9**. One of the possible explanations for this observation is that **9** associates with the active site of lipase differently to that of **1**, which contains an oxygen at the stereocenter. The

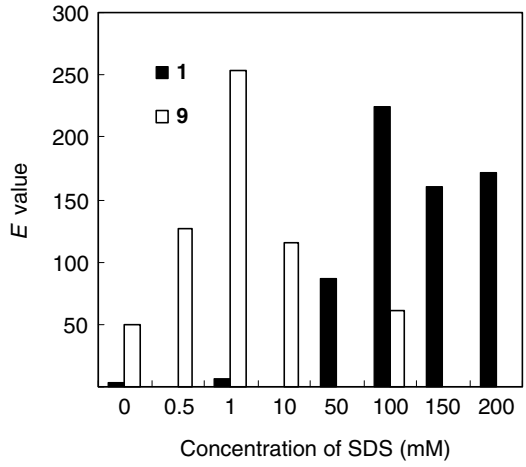

Figure 1. Variation of the enantioselectivity of the lipase-catalyzed hydrolysis of **1** and **9** by addition of SDS (0–200 mM).

scope of this enhancement effect of SDS on the enantioselectivity was evaluated by submitting the other substrates **2**–**8** to the lipase-catalyzed hydrolysis (Table 1). Inspection of the data summarized in Table 1 reveals that the remarkable enhancement of the *E* value brought

Table 1. Enhancement of the enantioselectivity by the addition of SDS for the lipase-catalyzed hydrolysis of **1**–**9** in an aqueous buffer solution

Ester	Additive	Time (min)	Conv. (%)	ee_p (%)[a]	*E*
1	None	8	36	50	4
	100 mM SDS	18	45	98	225
2	None	5	37	16	2
	100 mM SDS	330	29	88	22
3	None	5	40	30	2
	100 mM SDS	10	43	98	186
4	None	20	39	51	4
	100 mM SDS	20	45	97	123
5	None	15	35	28	2
	100 mM SDS	20	19	81	11
6	None	7	39	40	3
	100 mM SDS	10	44	98	122
7	None	4	45	38	3
	100 mM SDS	5	39	95	70
8	None	1080	25	86	18
	1 mM SDS	1080	23	>99	>500
9	None	660	42	92	49
	1 mM SDS	720	44	98	254

[a] Enantiomeric excess of the product.

Table 2. Effects of SDS on the enantioselectivity of the hydrolysis of **1** catalyzed by five different lipases

Lipase	Additive	Time (min)	Conv. (%)	% ee[a]	*E*
MY	None	8	36	50	4
	1 mM SDS	2.5	43	61	6
	100 mM SDS	18	45	98	225
OF	None	0.8	38	41	3
	1 mM SDS	0.1	40	70	10
	100 mM SDS	0.1	46	88	37
AY	None	2	25	49	3
	1 mM SDS	0.1	43	94	70
	100 mM SDS	0.05	43	97	130
PS	None	60	43	43	3
	1 mM SDS	30	40	65	7
	100 mM SDS	125 h	10	26	2
AH	None	90	39	65	7
	1 mM SDS	90	42	87	26
	100 mM SDS	47 h	5	55	4

[a] Enantiomeric excess of the product.

about by the action of SDS was observed for all the substrates. An increase in the *E* value up to two orders of magnitude was observed for some substrates examined. Furthermore, to test the generality of this SDS effect, the hydrolysis of **1** catalyzed by the other popular lipases was also carried out in the presence of SDS (1 and 100 mM) (Table 2). As can be seen from the *E* value listed in Table 2, this SDS effect is not limited to lipase MY. Among the lipases used, lipase AY and lipase OF from *C. rugosa* displayed almost the same enhancement effect in the *E* value as that for lipase MY. Lipase PS and lipase AH from *Pseudomonas cepacia* also moderately increased the *E* value, although the optimum concentration of SDS is different from that for lipase MY. The mode of the enhancement of the enantioselectivity and the optimum concentration of SDS seems to depend on both the structure of the substrate and the origin of lipase.

2.2. Effects of SDS on the structure of lipase and the initial rate of each enantiomer of the substrate

First, a possible conformational change of lipase MY caused by the addition of SDS was investigated on the basis of the results of FT-IR and fluorescence spectra. All the IR bands in the amide III region of lipase MY in an aqueous buffer solution with and without the SDS addition were assigned to individual secondary structural elements (Table 3), according to the known method of Gaussian curve fitting.[5] The α-helix content of 35% and the β-sheet of 16% (see Table 3) calculated from the areas of the IR bands of lipase MY in an aqueous buffer solution agree with the 33% α-helix and 12% β-sheet determined from its X-ray crystallographic data.[6] Furthermore, it was found by a comparison of the data in Table 3 that both IR spectra obtained in the presence and absence of SDS had essentially the same secondary structure composition. Additionally, the far-UV CD spectra of lipase MY in an aqueous buffer solution showed little change in the relative intensity of the negative band at 222 nm due to the α-helix structure after the addition of SDS. These facts mean that the native secondary structure of lipase MY is held steady

Table 3. Effects of SDS on the secondary structure of lipase MY determined by FT-IR in the amide III spectral region

Additive	Position (cm^{-1})	Assignment	Area (%)
None	1316	α	35
	1301	α	
	1287	α	
	1273	Random	48
	1259	Random	
	1244	Random	
	1229	β	16
100 mM SDS	1315	α	33
	1304	α	
	1290	α	
	1275	Random	50
	1260	Random	
	1245	Random	
	1230	β	17

even in the presence of 100 mM SDS. On the other hand, fluorescence spectra showed a marked decrease of intensity due to the emission of Trp and/or Tyr residues and a somewhat red shift of the maximum wavelength (Fig. 2), upon addition of SDS. The change of the fluorescence spectra indicates that Trp and/or Tyr residues in lipase MY are in a less hydrophobic environment when compared with those in the absence of SDS, with some conformational change, thus resulting in an increase of the flexibility of the lipase. This spectral result is also supported by our previous ESR study[2] in which the flexibility of lipase in organic solvents is affected by the addition of aqueous SDS.

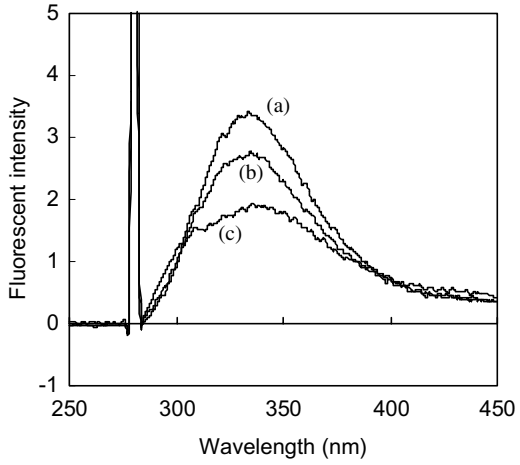

Figure 2. Fluorescence spectra of lipase MY dissolved in an aqueous buffer solution containing (a) 0 mM SDS, (b) 1 mM SDS, and (c) 100 mM SDS.

Next, to gain further insight into the origin of the enantioselectivity enhancement triggered by the SDS addition, we investigated the initial rate of reaction of each enantiomer of **1** for lipase MY-catalyzed hydrolysis (Table 4). Upon the addition of 100 mM SDS, the initial rate (V_R) of the correctly binding (*R*)-enantiomer was accelerated, whereas that (V_S) of the incorrectly binding (*S*)-enantiomer was seriously decelerated, when compared with no additive conditions. As can be seen from

the larger value of the quotient of the initial rates (V_R/V_S) listed in Table 4, the larger difference in the initial rates between the two enantiomers, arising from the opposite direction of each initial rate, is responsible for the significant enhancement of the enantioselectivity. In other words, judging from the results so far obtained, the enantioselectivity enhancement can be explained by assuming that some conformational change and/or an increase in the flexibility of lipase MY allow an easier access to its active site for the correctly binding (*R*)-enantiomer, but not for the incorrectly binding (*S*)-enantiomer.

Table 4. Effects of SDS on the initial rate of hydrolysis of each enantiomer of **1**

Additive	Initial rate (μmol h^{-1} mg^{-1})		V_R/V_S	*E*
	V_R[a]	V_S[a]		
None	13.4	1.72	8	4
100 mM SDS	44.5	0.22	200	225

[a] The V_R and V_S denote the initial rates of the (*R*)- and (*S*)-enantiomers, respectively.

3. Conclusions

The technique of SDS addition provides a useful method to improve the enantioselectivity of the lipase-catalyzed hydrolysis. This method is also appealing to organic chemists, because of its simplicity. Furthermore, the enantioselectivity enhancement brought about by SDS addition was found to be dependent on the acceleration of the initial rate of the correctly binding (*R*)-enantiomer and the deceleration of that for the incorrectly binding (*S*)-enantiomer.

4. Experimental

4.1. General

Lipases MY and OF and lipases AY, PS, and AH were generously provided by Meito Sangyo Co., Ltd, Japan, and Amano Pharmaceutical Co., Ltd, Japan, respectively. These lipases were used without further purification for the hydrolysis. For the spectral measurements, lipase MY was semi-purified by dialyzing and lyophilizing from crude material. The MALDI-TOF MS spectrum of the semi-purified lipase showed a single parent peak, *m*/*z* 60.2 kD, which is consistent with the molecular weight of *C. rugosa* lipase.[7] Ibuprofen and naproxen were purchased from Tokyo Kasei Kogyo Co., Ltd, Japan. All other chemicals were from commercial sources and of reagent grade. The FT-IR measurements were performed on a BRUKER TENSOR 27 spectropolarimeter. All the spectra were averaged over a total of 100 scans at 2 cm^{-1} resolution. The fluorescence spectra were recorded on a Shimadzu RF-5300 PC spectrofluorophotometer with an excitation wavelength of 280 nm and a bandwidth of 1.5 nm. The ^1H NMR spectra were recorded on a Varian Unity instrument (400 MHz) using $CDCl_3$ as a solvent.

4.2. Lipase-catalyzed hydrolysis, HPLC analysis, and determination of the enantiomeric ratio

In a typical procedure, racemic butyl esters **1**–**9** (0.036 mmol) were added to an aqueous buffer solution (2 mL), followed by the ultrasonic dispersion, and then lipase (30 mg) was added. At frequent intervals, aliquots were withdrawn and the supernatant analyzed by HPLC (a Shimadzu LC-10A) on a Chiralcel OK or a Chiralcel OD-H (Daicel Chemical Industries Co., Ltd, Japan) using an appropriate proportion of hexane/2-propanol as an eluent for each substrate. The enantiomers of the butyl esters **1**–**9** and hydrolysis products, the corresponding acids, were separated well enough for the accurate determination of the conversion and the enantiomeric excess (ee). The *E* value was calculated from the enantiomeric excess for the acid produced, according to the literature.[8] The results and conditions of HPLC analysis are shown in Table 5.

Table 5. HPLC characterization of the hydrolysis products

Substrate	Column	Eluent (hexane/2-propanol)[a]	Ret. time (min)[b]	
			R	*S*
1	Chiralcel OK	98/2	12.0	15.8
2	Chiralcel OK	98/2	15.4	18.8
3	Chiralcel OK	98/2	14.2	19.2
4	Chiralcel OK	98/2	10.2	14.1
5	Chiralcel OK	98/2	8.5	11.2
6	Chiralcel OK	98/2	37.4	56.9
7	Chiralcel OK	98/2	9.2	12.5
8	Chiralcel OD-H	99/1	19.1	22.5
9	Chiralcel OD-H	94/6	8.8	10.1

[a] All the eluents contain 0.1 vol % trifluoroacetic acid.
[b] Retention time of the hydrolysis product (the corresponding acid).

4.3. Measurement of the initial rate of each enantiomer of 1

In a typical procedure, (*R*)- or (*S*)-**1** (0.018 mmol) was added to an aqueous buffer solution (1 mL), followed by the ultrasonic dispersion after which lipase (15 mg) was added. The reaction mixture was then shaken (170 strokes min^{-1}) at 37 °C. At appropriate times, aliquots were withdrawn and the supernatant analyzed by HPLC on a chiral column to obtain the conversion. Several data points were collected to determine the initial rate. Each enantiomer used was prepared by the lipase-catalyzed esterification, according to our method.[9]

4.4. Preparation of racemic butyl esters 1–9

Racemic butyl esters **1**–**9** were prepared by the esterification of the corresponding acids with 1-butyl alcohol in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl). The corresponding, 2-aryloxypropanioc acids, were also prepared according to the known method.[10]

Butyl 2-(4-ethylphenoxy)propanoate (*RS*)-**1**. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, $J = 7.4$ Hz), 1.12 (3H, t, $J = 7.6$ Hz), 1.30 (2H, m), 1.59 (5H, m), 2.58 (2H, q,

$J = 7.6$), 4.15 (2H, m), 4.72 (1H, q, $J = 6.8$ Hz), 6.80 (2H, d, $J = 8.8$ Hz), 7.09 (2H, d, $J = 8.4$ Hz).

Butyl 2-phenoxypropanoate (*RS*)-**2**. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, $J = 7.4$ Hz), 1.29 (2H, m), 1.60 (5H, m), 4.15 (2H, m), 4.76 (1H, q, $J = 6.8$ Hz), 6.87 (2H, m), 6.97 (1H, m), 7.27 (2H, m).

Butyl 2-(4-methylphenoxy)propanoate (*RS*)-**3**. ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, $J = 7.4$ Hz), 1.31 (2H, m), 1.60 (5H, m), 2.27 (3H, s), 4.15 (2H, m), 4.71 (1H, q, $J = 6.4$ Hz), 6.77 (2H, d, $J = 8.8$ Hz), 7.06 (2H, d, $J = 8.8$ Hz).

Butyl 2-(4-*n*-propylphenoxy)propanoate (*RS*)-**4**. ^{1}H NMR ($CDCl_3$): δ 0.90 (6H, m), 1.29 (2H, m), 1.59 (7H, m), 2.51 (2H, t, $J = 7.6$ Hz), 4.15 (2H, m), 4.72 (1H, q, $J = 6.8$ Hz), 6.79 (2H, d, $J = 8.0$ Hz), 7.06 (2H, d, $J = 8.4$ Hz).

Butyl 2-(4-*t*-Butylphenoxy)propanoate (*RS*)-**5**. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, $J = 7.4$ Hz), 1.29 (11H, m), 1.59 (5H, m), 4.16 (2H, m), 4.72 (1H, q, $J = 6.4$ Hz), 6.80 (2H, d, $J = 9.2$ Hz), 7.27 (2H, d, $J = 9.2$ Hz).

Butyl 2-(4-methoxyphenoxy)propanoate (*RS*)-**6**. ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, $J = 7.4$ Hz), 1.31 (2H, m), 1.59 (5H, m), 3.76 (3H, s), 4.15 (2H, m), 4.66 (1H, q, $J = 6.8$ Hz), 6.82 (4H, m).

Butyl 2-(4-trifluorophenoxy)propanoate (*RS*)-**7**. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, $J = 7.4$ Hz), 1.27 (2H, m), 1.61 (5H, m), 4.16 (2H, m), 4.80 (1H, q, $J = 6.8$ Hz), 6.93 (2H, d, $J = 8.8$ Hz), 7.53 (2H, d, $J = 9.0$ Hz).

Butyl 2-(4-isobutylphenyl)propanoate (*RS*)-**8**. ^{1}H NMR ($CDCl_3$): δ 0.90 (9H, m), 1.28 (2H, m), 1.52 (5H, m), 1.84 (1H, m), 2.44 (2H, d, $J = 7.2$), 3.68 (1H, q, $J = 7.2$ Hz), 4.06 (2H, t, $J = 6.6$ Hz), 7.08 (2H, d, $J = 8.0$ Hz), 7.23 (2H, d, $J = 8.0$ Hz).

Butyl 2-(6-methoxy-2-naphthyl)propanoate (*RS*)-**9**. ^{1}H NMR ($CDCl_3$): δ 0.86 (3H, t, $J = 7.4$ Hz), 1.29 (2H, m), 1.56 (5H, m), 3.84 (1H, q, $J = 6.4$ Hz), 3.91 (3H, s), 4.08 (2H, m), 7.13 (2H, m), 7.42 (1H, d, $J = 8.8$ Hz), 7.69 (3H, m).

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