A Dramatic Improvement of Enantioselectivity of Lipase in Organic Solvents by Addition of Aqueous SDS: A Close Correlation between Enantioselectivity and Conformational Flexibility of Lipase

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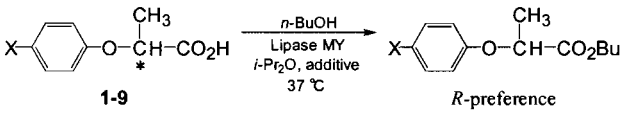
The enantioselectivity of the lipase-catalyzed esterification of 2-(4-substituted phenoxy)propanoic acids was found to be dramatically enhanced by addition of aqueous sodium dodecyl sulfate (SDS) as a new type of additive to the reaction medium, isopropyl ether. The origin of the enhanced enantiomeric differentiation may be attributed to the increased conformational flexibility of lipase triggered by the SDS action, the flexibility of which can be estimated from the changes in the ESR spectra observed for the mobility of a spin label bound to its active-site.

Lipases have been established as effective catalysts in organic solvents for the preparation of enantiomerically pure compounds.[1] For lipase-catalyzed reactions in organic solvents, however, their enantioselectivities are not always high to obtain chiral compounds in which only one of the enantiomers is biologically active. This is mainly because a non-natural substrate such as the pharmaceutical or the agricultural chemicals, in most case, is used for enzyme reactions performed by lipases in organic solvents. In other words, the rigid conformation of lipase in organic solvents may prevent its active site from accepting a non-natural substrate with a structure significantly different from that of a natural one.

In order to overcome this problem, organic chemists have often employed various additives in the reaction mixture, because it is a convenient way for improving the ability of enzymes, in comparison with the tedious procedure of finding a more enantioselective enzyme from other sources. In a recent review, various additives have been successfully applied to improve the enantioselectivity and reactivity for lipase-catalyzed reactions.[2]

Here, we wish to report the finding of aqueous SDS (sodium dodecyl sulfate) as a new type of additive that enhances dramatically the enantioselectivity in esterification of 2-(4-substituted phenoxy)propanoic acids in isopropyl ether by lipase MY from *Candida rugosa*. This choice of the additive is based on a view that the increased flexibility of lipase brought about by addition of SDS may be significantly associated with its induced-fit conformational adjustment for a non-natural substrate.

In a typical lipase-catalyzed reaction, the substrates **1**–**9** (0.36 mmol) and *n*-BuOH (1.08 mmol, 3 equiv) were dissolved in dry isopropyl ester (2 mL). To the solution, a small amount of aqueous SDS was added, followed by ultrasonic dispersion, and then lipase MY from *Candida rugosa* (30 mg) was added. The suspension was shaken (170 strokes/min) at 37 °C. The *E* value was calculated from the enantiomeric excess (ee) for the butyl ester produced, according to the literature.[3] The ee was measured by HPLC on a chiral column (Daicel Chiralcel OK).



Scheme 1. Lipase-catalyzed esterification of 2-(4-substituted phenoxy)propanoic acids with *n*-BuOH in isopropyl ether containing the additive.

For the lipase-catalyzed esterification of 2-(4-substituted phenoxy)propanoic acids as our model reaction (Scheme 1), the *R* enantiomer of the butyl ester was preferentially produced in all the substrates **1**–**9**. The ordinary reaction conditions without the additive, unfortunately, displayed the rather poor enantioselectivity; **1**(*E* = 3.8), **2**(*E* = 2.0), **3**(*E* = 1.6), **4**(*E* = 5.6), **5**(*E* = 4.8), **6**(*E* = 1.5), **7**(*E* = 1.4), **8**(*E* = 1.3), and **9**(*E* = 1.5). This result prompted us to test a new approach for improving the enantioselectivity by addition of aqueous SDS to isopropyl ether of the reaction medium. As is seen in Figure 1, the *E* values (as a measure of the enantioselectivity) for **1**–**9** at ca. 40% conversion in the model reaction are found to be dramatically enhanced by the addition of 1.2 vol% of 0.7 M SDS,[4] the effect of which is always much larger than that of water (1.2 vol%).

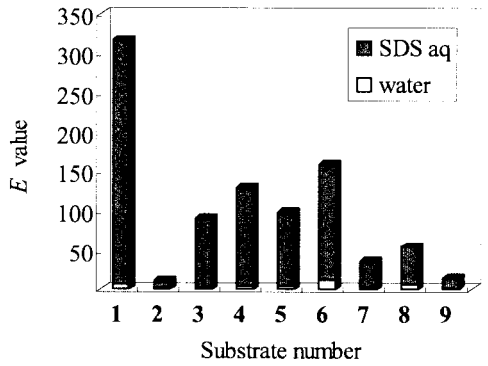


Figure 1. Enhancement of the enantioselectivity (*E* value) by addition of aqueous SDS (0.7 M, 1.2 vol%) for the lipase-catalyzed esterification of **1**–**9** in isopropyl ether.

One could speculate that the observed increase of the enantioselectivity is primarily attributed to the SDS-induced flexibility of lipase. Therefore, to investigate whether the enantioselectivity may be controlled by the conformational flexibility of lipase, the mobility of a spin label (1-oxy-2,2,6,6-tetramethyl-4-piperidinyl ethoxyphosphorofluoridate) bound to its active-site

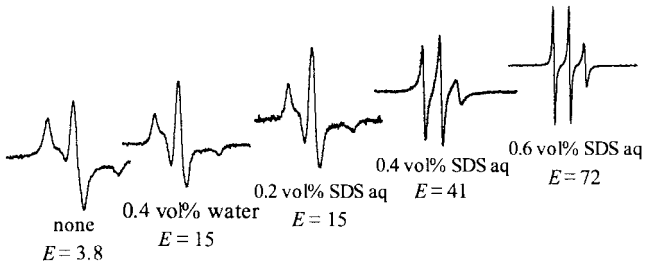


Figure 2. Relationship between the enantioselectivity for **1** and the conformational flexibility of lipase judged from the changes in the ESR spectra.

was examined at SDS contents by the measurement of the ESR spectrum. The results of the ESR spectra measured in isopropyl ether with 0, 0.2, 0.4, and 0.6 vol% of 5 mM SDS are summarized together with that of 0.4 vol% of water in Figure 2, which includes also the *E* value of the reaction carried out under the same additive condition. As is seen in Figure 2, as the SDS content increases, the three spectral lines are found to narrow in width and increase in peak height. This behavior is consistent with those of the ESR spectra observed for subtilisin Carlsberg suspended in tetrahydrofuran by addition of water[5] and in isooctane by addition of dimethyl sulfoxide.[6] This spectral change means that the conformation of the lipase's active-site surrounding the spin label becomes flexible.[5,7] It should be noteworthy that the *E* value for **1** increases with the increase of the lipase's flexibility judged from the changes in the ESR spectra (Figure 2). Interestingly, the additive conditions at both 0.2 vol% of SDS and 0.4 vol% of water exhibited the similar feature of the spectral lines and displayed almost the same *E* value (Figure 2). This fact again supports a close correlation between the conformational flexibility and the enantioselectivity of lipase.

Next, we have investigated the effect of the lipase's flexibility on relative V_{max} and/or K_m values for each enantiomer of **1** prepared according to our method[8] (Table 1). It can be seen in Table 1 that the presence of SDS, corresponding to the increased flexibility of lipase, brings about the significantly increased V_{max} and decreased K_m values for the *R* enantiomer, although both values for the *S* enantiomer are not sensitive to the additive. Also, the observed order in the value of catalytic efficiency (V_{max}/K_m) for the *R* enantiomer is as follows; SDS additive >> water additive > no additive (Table 1). Consequently, a larger flexibility of lipase may allow its easier access

Table 1. Kinetic parameters for the lipase-catalyzed esterification of each enantiomer of **1** in isopropyl ether under various additive conditions

Additive	Config.	K_m / mM	V_{max} / μ mol·h^{-1}	V_{max}/K_m / h^{-1}
None	*R*	289	0.0848	2.9×10^{-4}
	S	420	0.0811	1.9×10^{-4}
Water	*R*	232	8.39	3.6×10^{-2}
	S	397	3.65	9.2×10^{-3}
SDS aq	*R*	100	25.4	2.5×10^{-1}
	S	390	0.216	5.5×10^{-4}

through the induced-fit motion for the correct binding *R* enantiomer, in sharp contrast to that for the incorrect binding *S* enantiomer, thus resulting in the marked improvement of the enantioselectivity.

Our finding should be useful for understanding the mechanism for optimization of enzyme-catalyzed reactions in organic solvents.

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