CHEMCOMM Communication
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Reversible activity changes of a lipid-coated lipase for enantioselective esterification in supercritical fluoroform†

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Received (in Cambridge, UK) 5th June 2001, Accepted 7th August 2001
First published as an Advance Article on the web 4th September 2001

Enzymatic activity of a lipid-coated lipase for enantioselective esterification of (*R*)-1-phenylethanol in supercritical fluoroform can be reversibly controlled by changing pressure or temperature that reflects changes of dielectric constant.

We have reported that a lipid-coated lipase, in which hydrophilic head groups of lipids interact with the enzyme surface and two lipophilic long alkyl-chains extend away from its surface that solubilize the enzyme in organic solvents, acts as an efficient catalyst for enantioselective esterification (the reverse hydrolysis reaction).[1] The catalytic activity of the lipid-coated lipase was 20–100 times higher than that of other lipase systems, including enzymes in organic–aqueous emulsion,[2] enzyme powders dispersed in organic solvents,[3] and poly(ethylene glycol)-grafted lipase in organic solvents.[4] However, the activity and stability of the lipid-coated lipase in organic media were largely dependent on their polarity, as well as other enzyme systems: the esterification rate of the lipid-coated lipase was high in nonpolar isooctane or benzene, but denatured in polar media such as chloroform and THF.[1] This is a large disadvantage of enzyme reactions in organic media, because the polar solvents are attractive for the high reaction rates and the high solubility of substrates.

Recently, supercritical fluids (scFs) became attractive as a new medium for chemical reactions including enzymatic reactions, since their physical properties (*e.g.* polarity, diffusion, and viscosity) are intermediate between those of gases and liquids, and they can be manipulated by small changes in pressure or temperature.[5–7]

In this communication, we report that the reaction rate of the enantioselective esterification catalyzed by lipid-coated lα in supercritical fluoroform (scCHF$_3$) can be reversibly controlled by changing temperature or pressure (reflecting polarity changes) without denaturing enzymes. The reason fluoroform was chosen as the scF is that the dielectric constants (ε) of scCHF$_3$ can be controlled from 1 to 7 (corresponding to the ε values of hexane to THF as organic media) by changing either temperature or pressure of scFs, although ε values of scCO$_2$ could be changed only in the narrow range of 0.1–0.3.

A lipid-coated lipase B (from *Pseudomonas fragi*) was prepared by mixing aqueous solutions of enzyme and lipid molecules in the same way as reported previously.[1,7] The lipid-coated enzyme was found to be soluble (*ca.* 1 mg/10 mL) in scCHF$_3$ in the range 30–60 °C and 50–150 atm, by observation with a pressure-resistant glass vessel (Taiatsu Techno, Co., Tokyo, volume: 10 mL), but not very soluble in liquid CHF$_3$ (at 20 °C with 60 atm) and insoluble in gaseous CHF$_3$ (at 40 °C with 40 atm).

Enantioselective esterifications were carried out as follows.[1,7a] In a stainless steel or pressure-resistant glass vessel both substrates of (*R*)- or (*S*)-1-phenylethanol, lauric acid and a lipid-coated lipase B were added, then liquid CHF$_3$ was injected at 50–150 atm from a LC pump (Jasco PU-980 HPLC pump) connected to a CHF$_3$ gas cylinder. The vessel was warmed under stirring magnetically above T_c = 26 °C to create a supercritical state, and the pressure was kept constant (±0.1 atm) by a backpressure regulator. At every appointed time, the vessel was degassed carefully under cooling at 0 °C. The residual powder was solubilized in CH$_3$CN and analyzed by a HPLC.

Fig. 1 shows typical time courses of the enantioselective esterification of 1-phenylethanol (50 mM) with lauric acid (100 mM) catalyzed by a lipid-coated lipase B (1 mg of protein) in scCHF$_3$ at 40 °C with 60 atm. The lipid-coated lipase B can catalyze enantioselelctively the esterification of the (*R*)-alcohol but not the (*S*)-alcohol at 60% conversion, and the reactivity of racemic alcohols are in between (*R*)- and (*S*)-isomers. The initial rate for the (*R*)-isomer and the enantioselectivity were similar to those obtained in non-polar organic solvents such as isooctane.

One of the features of scF as reaction media is that the physicochemical properties such as dielectric constant (ε) of media can be continuously changed by varying temperature or pressure in the scF state.[5] A phase diagram of CHF$_3$ is shown in the contents list entry for this communication.† Effects of continuous changes of temperature or pressure on the initial rate, enantioselectivity (v_R/v_S), and conversion of the esterification catalyzed by a lipid-coated lipase B are shown in Fig. 2. When pressures were continuously changed at the constant temperature of 40 °C (Fig. 2a), reaction rates were slow below P_c = 48 atm, where the medium exists as gaseous CHF$_3$ and the lipid-coated enzymes exist as powders. Reaction rates for the

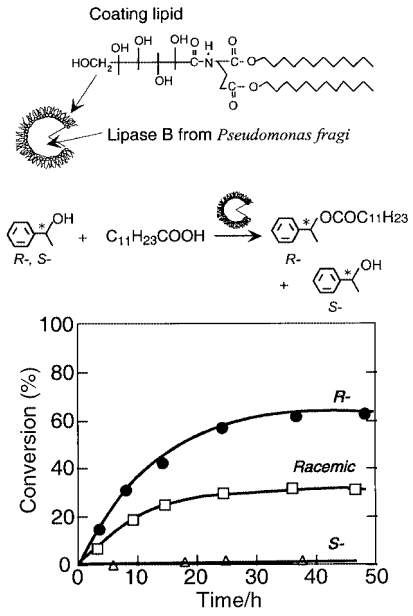

Fig. 1 A schematic illustration of a lipid-coated lipase B and time-courses of enantioselective esterification of 1-phenylethanol (50 mM) and lauric acid (100 mM) catalyzed by the lipid-coated lipase B (1 mg of protein) in 10 mL of scCHF$_3$ at 40 °C with 60 atm.

† Electronic supplementary information (ESI) available: Table S1 and phase diagram of CHF$_3$. See http://www.rsc.org/suppdata/cc/b1/b104913p/

 DOI: 10.1039/b104913p

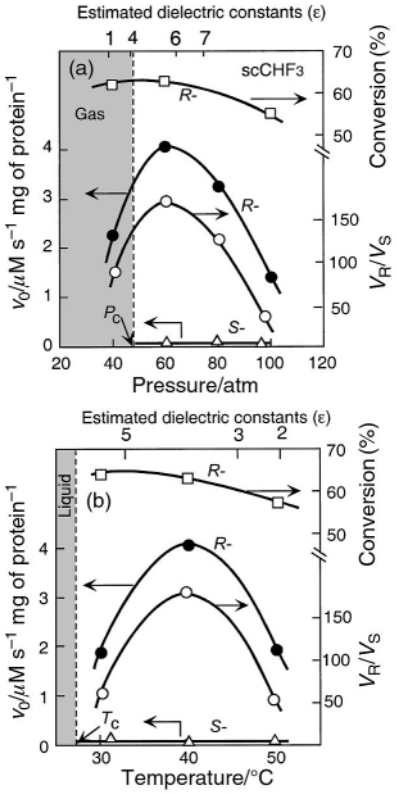

Fig. 2 Effect of (a) pressure changes at 40 °C and (b) temperature changes with 60 atm on the initial rate, enantioselectivity (v_R/v_S), and conversion of the esterification of 1-phenylethanol catalyzed by the lipid-coated lipase B in $scCHF_3$.

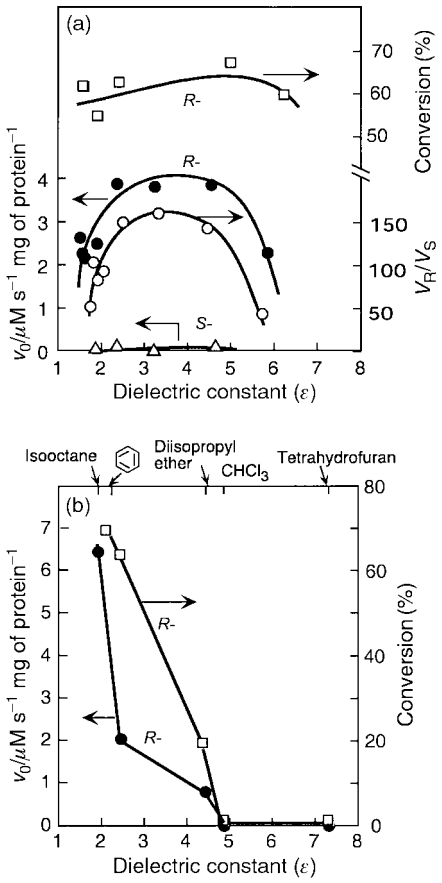

Fig. 3 The initial rate, enantioselectivity, and conversion of the esterification were plotted against dielectric constants (ε). (a) In the $scCHF_3$ obtained by pressure and temperature changes, and (b) in the conventional organic solvents by changing media.

(*R*)-isomer showed a maximum near 60 atm and then decreased with increasing pressure to 100 atm, where the lipid-coated enzyme is homogeneously solubilized in $scCHF_3$. However, the conversions for the (*R*)-isomer were constant (60 ±5%) in the range of 40 to 100 atm. The reaction rates for the (*S*)-isomer were very low in all pressure ranges. The enantioselectivity also showed a bell-shaped curve mainly due to the reactivity change of the (*R*)-isomer. A similar tendency was observed when temperatures were changed from 30 to 50 °C at the constant pressure of 60 atm: the initial rate for the (*R*)-isomer and the enantioselectivity showed a bell shape behavior, although the conversion was constant (60 ±5%) (Fig. 2b). The reaction rates for the (*S*)-isomer were very low in all temperature ranges. When the temperature or pressure of $scCHF_3$ was changed continuously, it is well known that dielectric constants (ε) of the media altered continuously[5] and these estimated ε values are shown at the upper *x*-axis of Fig. 2.

In Fig. 3a, the initial rare, enantioselectivity, and conversion are plotted against apparent ε values at different pressures (40–100 atm) and temperatures (30–50 °C) of $scCHF_3$. The initial rates for the (*R*)-isomer showed a bell-shaped curve and the conversion for the (*R*)-isomer was constant and independent of ε values of $scCHF_3$. The enantioselectivity also showed a bell-shaped curve due to the change in reactivity of the (*R*)-isomer. In Fig. 3b, the results obtained in the conventional organic solvents are plotted against ε values of organic solvents, in comparison. Both the initial rate and conversion for the (*R*)-isomer drastically decreased with increasing polarity of the solvents. Thus, the enzyme is active in the non-polar solvents such as isooctane and benzene, however, it easily denatured in the polar solvent and did not revert the reactivity. When $scCHF_3$ was employed as the medium, the polarity could be altered continuously by changing pressure or temperature without changing media, and the reaction rate could be controlled reversibly with keeping the high conversion. Thus, the enzyme can be kept inactive at 30 °C with 40 atm or 50 °C with 100 atm, and be reverted to active at 40 °C with 60 atm in $scCHF_3$. This could be controlled reversibly for at least 10 cycles.

In conclusion, the lipid-coated lipase is soluble and can catalyze the esterification enantioselectively and the reaction rate could switch on and off by adjusting pressure or temperature of $scCHF_3$. The enantioselectivity was also affected by pressure or temperature changes. We believe that the combination of the lipid-coated enzyme and $scCHF_3$ as reaction media will become a new system in biotransformation studies.

Notes and references

1 T. Mori and Y. Okahata, *Methods in Biotechnology*, vol. 15, *Enzymes in Non-aqueous Solvents, Methods and Protocols*, ed. E. N. Halling and H. L. Holland, Humana Press, Inc., Totowa, NJ, 2001, ch. 9, p. 83–94; Y. Okahata, Y. Fujimoto and K. Ijiro, *J. Org. Chem.*, 1995, **60**, 2244; Y. Okahata and T. Mori, *Trends Biotechnol.*, 1997, **15**, 50.

2 P. L. Luisi, *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 439.

3 A. M. Klibanov, *Trends Biochem. Sci.*, 1989, **14**, 141.

4 Y. Inada, T. Yoshimoto, T. Matsushima and Y. Saito, *Trends Biotechnol.*, 1986, **4**, 67.

5 A. Clifford, *Supercritical Fluids, Fundamentals for applications*, NATO ASI Ser. E, **273**, pp. 449–479, Kluwer Academic, Netherlands, 1994; S. Angus, B. Armstrong and K. M. de Reuck, Carbon Dioxide, International Thermodynamic Tables of the Fluid State, ver. 3, pp. 13–23, Pergamon, New York, 1996.

6 K. Nakamura, Y. M. Chi, Y. Yamada and T. Yano, *Chem. Eng. Commun.*, 1986, **45**, 207.

7 (*a*) T. Mori, A. Kobayashi and Y. Okahata, *Chem. Lett.*, 1998, 921; (*b*) T. Mori and Y. Okahata, *Chem. Commun.*, 1998, 2215.