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Control on enantioselectivity with pressure for lipase-catalyzed esterification in supercritical carbon dioxide

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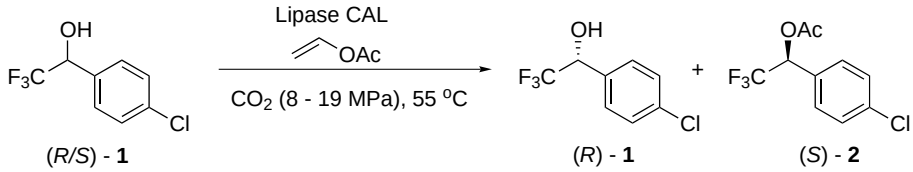
Abstract—The enantioselectivity of lipase-catalyzed esterification of 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol in supercritical carbon dioxide changed *continuously* from $E=10$ to 50 by decreasing the pressure at 55°C. The effect of the solvent on enantioselectivity was examined without changing the kind of solvent.

Supercritical fluids are of continuing interest and importance as tunable solvents and reaction media in the chemical process industry.[1] They are neither gas nor liquid but can be compressed gradually from low to high density. By adjusting the density, the properties of these fluids can be customized and manipulated for a particular process.[1] Among many fluids, supercritical carbon dioxide ($scCO_2$) has the advantages of an environmentally benign nature, non-flammability, low toxicity, high availability, and ambient critical temperature ($T_c=31.0$°C).[1] Several recent publications have demonstrated the potential of $scCO_2$ as an alternative reaction medium to toxic organic solvents for synthetic transformations with transition metal catalysts.[2,3] Our interest is in the reaction by a natural catalyst, biocatalyst, for synthesizing optically pure fluorinated alcohols that have received much attention for the synthesis of ferroelectric liquid crystals or bioactive fluorinated compounds.[4–7] Mori et al.[8,9] have demonstrated the benefit of using $scCO_2$ for biotransformations, for example improved and controllable reaction rates. We examined the enantioselective acetylation of racemic 1-(*p*-chlorophenyl)-2,2,2-trifluoroethanol (*R*/*S*)-**1** with the lipase CAL (Novozym 435) and vinyl acetate[10] in CO_2, affording (*S*)-acetate (*S*)-**2** and remaining (*R*)-alcohol (*R*)-**1**, as shown in Scheme 1. We found that the enantioselectivity of the reaction can be tuned *continuously* from $E=10$ to 50 by adjusting the pressure of CO_2 at 55°C. The effect of the solvent was examined without changing the kind of solvent.

For the acetylation of (*R*/*S*)-**1** with the lipase in CO_2, we used a high pressure-resistant stainless-steel vessel.[11] In the vessel, the lipase (CAL, 25 mg), the racemic alcohol (*R*/*S*)-**1** (2.5 mg, 0.012 mmol), vinyl acetate (0.025 mL, 0.27 mmol), and a magnetic stirrer bar were charged (the chemicals were placed in a glass tube to prevent them from contacting the biocatalyst before achieving supercritical conditions). Then the vessel was warmed to 55°C, and CO_2, preheated to 55°C, was introduced until a desired pressure (8–19 MPa, 1 MPa = 9.87 atm) was reached. The mixture was then



Scheme 1.

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stirred at 55°C for the desired time (2–4 h) and worked up as described.[11] The ee of both the remaining alcohol **1** and the producing acetate **2** were measured with a chiral GC column (Chirasil-DEX CB; 25 m; He 2 mL/min, 130°C for 6 min followed by 10°C/min to 180°C) to determine the yields and *E* values, the ratio of the specificity constants.[12] The absolute configurations were determined by comparing the GC retention times with those of authentic samples.[13]

Many hydrolytic enzymes have been reported to show activities in supercritical solvents,[8,9,14–16] and, as expected, (*R*/*S*)-**1** was acetylated with the lipase CAL in CO_2 at 55°C under the pressure range of 8 MPa to 19 MPa. Below 8 MPa at 55°C, the reaction hardly proceeded, and the reaction above 19 MPa was not examined. The reaction yield of acetate **2** reached around 50% in 5 h at 10 MPa (Fig. 1).

The effect of pressure on enantioselectivity was investigated. As shown in Fig. 2, the *E* value changed *continuously* from 50 to 10 when the pressure was changed from 8 to 19 MPa, regardless of the reaction time.

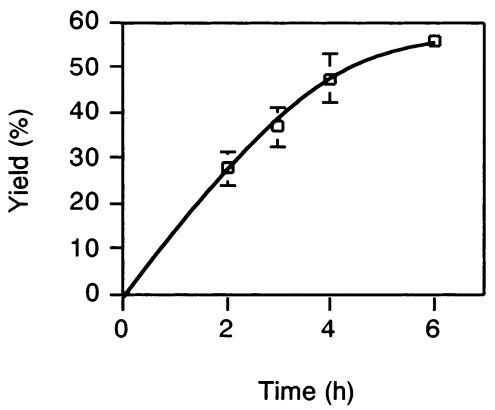

Figure 1. Time course of acetylation catalyzed by lipase at 10 MPa.

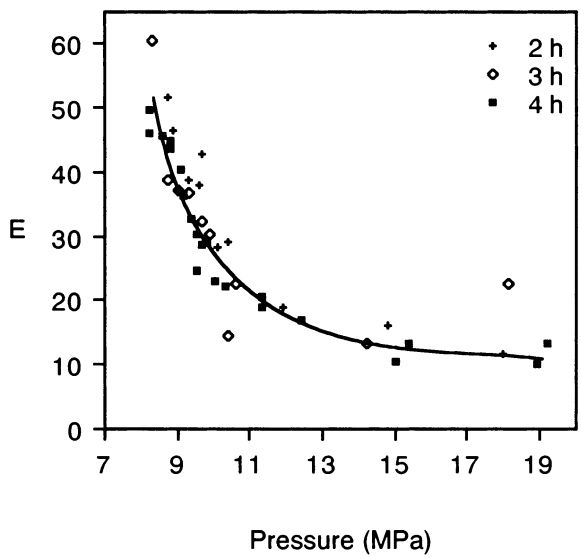

Figure 2. Effect of pressure on enantiomeric ratio (*E*).

Contrarily, the continuity of the enantioselectivity change was not observed using conventional organic solvents for the same reaction (the same enantiomer (*S*)-**1** reacted faster). As shown in Fig. 3, *E* values were largely affected by the solvents used. However, it is ambiguous whether the *E* values depended only on the polarity[17] of the solvent, because the polarity change is inevitably accompanied by the change in the molecular structure of the solvent (cyclic or acyclic). This result agrees with the literature report that the molecular structure of the solvent, besides its polarity, affects the enantioselectivity of the reaction.[18] On the other hand, by using CO_2, the solvent properties can be changed by the manipulation of pressure, without changing the kind of solvent.

The tunable enantioselectivity of the reaction is indeed noteworthy, but the cause is not clear at present. A search of the literature uncovered several reports that investigated the effect of solvent properties on enzyme enantioselectivities.[19–25] Kamat[19] attributed the change in the enantioselectivity of Subtilisin carlsberg and an *Aspergillus* protease by manipulation of the pressure of supercritical fluoroform to the change in the polarity of the fluoroform.[26] However, in our work, the change in the pressure of CO_2, rather than fluoroform, does not cause a significant change in the polarity evaluated as dielectric constant (at 50°C, 1.12 at 8 MPa and 1.29 at 11 MPa)[27] and log *P* (at 50°C, 1.4 at 8 MPa and 1.9 at 11 MPa).[28] It is not clear if this small change in polarity has a large effect on this reaction. On the other hand, the density of CO_2 changes from 0.20 to 0.42 kg/L by changing the pressure from 8 to 11 MPa at 55°C.[29] Ikushima explained the high enantioselectivity of lipase in a very limited pressure range at 304.1 K by the interaction between CO_2 and enzyme molecules.[21–23] We also propose that the large change in density could

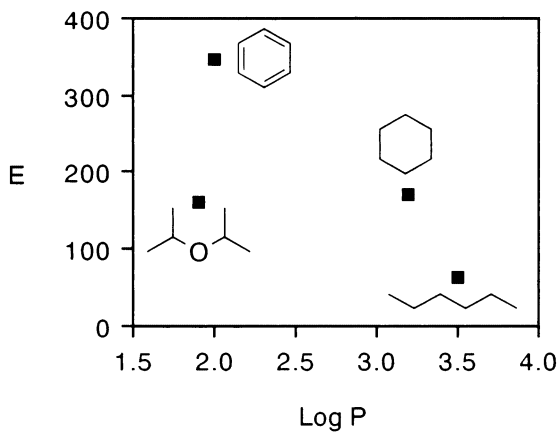

Figure 3. Effect of organic solvent on enantiomeric ratio (*E*). *Reaction conditions*: Substrate: 7.7 μmol, vinyl acetate: 88 μmol, lipase: 20 mg for reaction in hexane and cyclohexane, 50 mg for benzene and isopropyl ether, molecular sieves 4 Å: 50 mg, organic solvent: 500 μL, 130 rpm, 55°C, 2 h for hexane and cyclohexane, 2.5 h for benzene, 4 h for isopropyl ether. The reaction was stopped at the conversion of 50% for hexane, 47% for cyclohexane, 47% for benzene and 39% for isopropyl ether.

significantly change the interaction of CO_2 and enzyme, causing adsorption of CO_2 on the enzyme as reported in other proteins[30] and/or incorporation of CO_2 in the substrate-binding pocket of the enzyme as reported in the incorporation of organic molecule in the enzymes.[18,24] These interactions may change the conformation of the enzyme gradually corresponding to the pressure, resulting in a *continuous* change in enantioselectivity. However, further investigation is necessary to clarify the mechanism.

In conclusion, the enantioselectivity of an environmentally benign reaction pairing the natural catalyst and natural solvent was examined, and the *continuous* change in enantioselectivity without changing the molecular structure of the solvent, which is not possible by simply changing the organic solvent, was observed using CO_2. We believe this reaction will be helpful not only for studying the origin of enantioselectivity but also for synthesis of useful compounds while keeping harmony with the natural environment.

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