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Novel strategic lipase-catalyzed asymmetrization of 1,3-propanediacetate in supercritical carbon dioxide

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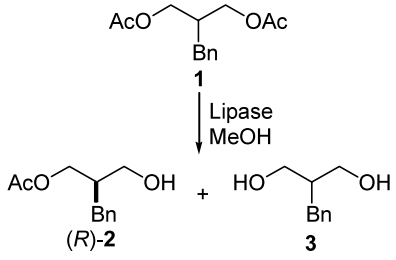
Abstract—In lipase-catalyzed asymmetrization of 1,3-propanediacetate no enantioselectivity was observed in conventional organic solvents, whereas in supercritical carbon dioxide ($scCO_2$) enantioselectivities were observed up to 50% ee, which probably arose from a conformational changing of lipase at the active site due to a transformation of the amino group of lysine into carbamic acid.

Recently, supercritical fluids (scF) have been focused on as a new reaction medium.[1] Enzymes have become one of the most useful catalysts under scF conditions in the past two decades,[1,2] especially, hydrolytic enzymes such as lipase and protease are intensively investigated.[3,4] Advantages of using scF in an enzymatic reaction are as follows: (i) increasing the rate of mass-transfer, (ii) simple separation of the product and (iii) environmentally benign reaction media, etc. Indeed, a large number of literature reported higher enzyme activity and a greener chemical process than those in conventional organic media.[5] With respect to stereoselectivity, however, most literature investigated enzymatic reactions that had shown high stereoselectivity in conventional organic media. An advantage of using scF for stereoselectivity has not been clarified. In this paper, we report a novel enhancement of stereoselectivity caused by a conformational changing of lipase in supercritical carbon dioxide ($scCO_2$).

Lipase-catalyzed asymmetrization of 2-benzyl-1,3-propanediacetate (**1**) was examined as a model reaction (Scheme 1). Kinetic resolution affords only a maximum of 50% product yield, while asymmetrization is theoretically possible to give 100% yield of the product. In addition, the chiral monoacetate **2** obtained is an important chiral building block for the synthesis of natural products.[6]

Before examining enzymatic transesterification in $scCO_2$, 15 commercially available lipases were studied to assess activities, chemoselectivities and enantioselectivities. To a solution of diacetate **1** (30 mg) in hexane, which showed approximately equal polarity and solubility as $scCO_2$, were added methanol (2 equiv.) and lipase (30 mg). The suspension was stirred for 24 h at 40°C, filtered and analyzed with HPLC (CHIRALPAK AS) with hexane/ethanol as a solvent. The best conversion was achieved with CHIRAZYME® L-2, c.-f., lyo (*Candida antarctica*, Type B); however, no enantioselectivity was observed (Table 1, entry 1). Similarly, the other 14 lipases showed low enantioselectivities (0–59% conversion, 0–15% ee). The effect of conventional organic solvents showed a well-known tendency, that is, a hydrophilic organic solvent such as methanol is generally an inferior solvent than hydrophobic ones (Table 1). It was obvious that lipase-catalyzed asymmetrization of the diacetate **1** showed almost no enantioselectivity in conventional organic media.

Lipase-catalyzed asymmetrization of the diacetate **1** in $scCO_2$ was carried out as follows: In a stainless steel pressure-resistant vessel (Taiatsu Techno, Co., TVS-N2 type, 10 mL), lipase (30 mg) and methanol (10 equiv.) were added. To the vessel the diacetate **1** (30 mg),



Scheme 1. Lipase-catalyzed asymmetrization of the 1,3-propanediacetate **1**.

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Table 1. CHIRAZYME® L-2-catalyzed asymmetrization of the diacetate **1** in conventional organic solvent[a,b]

Entry	Solvent	Conversion (%)	**2** yield (%)	ee (%)	**3** yield (%)
1	Hexane	68	59	0	9
2	Toluene	78	54	0	24
3	*i*-Pr_2O	91	56	0	35
4	1,4-Dioxane	67	27	7	40
5	MeOH	10	8	6	2

[a] Reactions were carried out in the presence of methanol (2 equiv.) and CHIRAZYME® L-2 (1 g equiv.) at 40°C for 24 h.
[b] The yields and the enantiomeric excesses were determined by HPLC analysis.

which was put into a micro tube to avoid direct contact with lipase, was added. The vessel was sealed and warmed to appropriate temperature, then liquid CO_2 was injected under appropriate pressure using a pump (AKICO supercritical fluid system). The reaction was stirred with a magnetic stirrer. On completion of the reaction, the vessel was cooled to 0°C with an ice bath and pressure was released slowly to atmospheric pressure. The residual mixture was filtered, washed with ether and analyzed with HPLC.

Time course for the asymmetrization of the diacetate **1** carried out in $scCO_2$ at 10 MPa and 40°C is shown in Table 2. Interestingly, enantioselectivities were observed. The maximum enantiomeric excess (49% ee) was obtained at a reaction time of 3 h. The conversion increased along with the reaction time, which revealed that CHIRAZYME® L-2 catalyzed the asymmetrization even in $scCO_2$.

The effect of temperature was examined at constant pressure (10 MPa, 3 h), and the results are shown in Table 3. Under critical temperature (<31°C), the diacetate **1** did not react (entry 1). At 40°C the reaction rate and the enantiomeric excess were simultaneously at maximum (49% ee). Above 40°C, they gradually decreased.

Many properties of supercritical fluids, such as density, diffusion and viscosity, were simply controlled by manipulation of pressure. The effect of pressure was examined at constant temperature (40°C, 3 h) and results are shown in Table 4. Under critical pressure (<7.4 MPa) the reaction did not occur. Below 9 MPa the transformation of the monoacetate **2** to the diol **3** accelerated, while above 10 MPa it decelerated.[7] Enantioselectivity slightlydepended on pressure (50% ee at 20 MPa). Interestingly, the formation of the diol **3** highly depended on pressure.

It is well known that the reaction media can change the activity, specificity and stereoselectivity of an enzyme.[8] The enantioselectivity would be ascribed to a conformational changing of CHIRAZYME® L-2 due to a transformation of the amino group of lysine into carbamic acid. The structure and amino acid sequence of this lipase have been elucidated by Jones,[9] in which nine lysines are included. They are located relatively outside; therefore, the amino groups of lysine were exposed to $scCO_2$ and transformed into the carbamic acids. In consequence, originally non-stereoselective lipase would conformationally change the active site under appropriate pressure to give rise to stereoselectivity (Fig. 1). This conformational changing probably occurred stepwise under different pressure, whereby the formation of the diol **3** highly depended on pressure.[10]

Table 2. Time course for the asymmetrization of the diacetate **1**[a]

Entry	Time (h)	Conversion (%)	**2** yield (%)	ee (%)	**3** yield (%)
1	1	10	9	27	1
2	3	22	16	49	6
3	6	30	19	47	11
4	12	41	17	42	24
5	24	58	16	39	42

[a] Reactions were carried out in the presence of methanol (10 equiv.) and CHIRAZYME® L-2 (1 g equiv.) at 40°C and 10 MPa.

Table 3. Effect of temperature for the asymmetrization of the diacetate **1**[a]

Entry	Temp. (°C)	Conversion (%)	**2** yield (%)	ee (%)	**3** yield (%)
1	25	0	0	0	0
2	30	2	2	23	0
3	35	13	11	47	2
4	40	22	16	49	6
5	45	13	11	47	2
6	50	9	8	42	1
7	60	1	1	35	0

[a] Reactions were carried out in the presence of methanol (10 equiv.) and CHIRAZYME® L-2 (1 g equiv.) at 10 MPa for 3 h.

Table 4. Effect of pressure for the asymmetrization of the diacetate **1**[a]

Entry	Pressure (MPa)	Conversion (%)	**2** yield (%)	ee (%)	**3** yield (%)
1	7	0	0	0	0
2	7.5	75	11	23	64
3	9	77	14	23	63
4	9.5	18	13	40	5
5	10	22	16	49	6
6	11	29	18	49	11
7	12	33	20	42	13
8	14	32	17	40	15
9	16	34	17	39	17
10	18	30	20	34	10
11	20	41	21	50	20

[a] Reactions were carried out in the presence of methanol (10 equiv.) and CHIRAZYME® L-2 (1 g equiv.) at 40°C for 3 h.

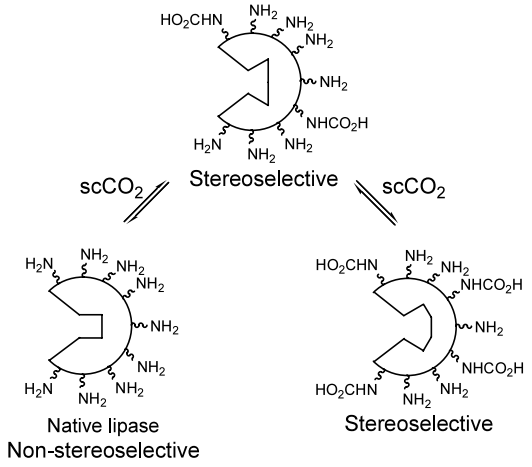

Figure 1. Proposed mechanism of enantioselectivity.

Direct observation of the conformational changing in $scCO_2$ is difficult at this stage, so we tried a different approach for observations. In a similar manner in Table 1, the reaction of **1** using treated-CHIRAZYME® L-2, which had been exposed to $scCO_2$ for 24 h before use, showed no enantioselectivity (Table 5, entry 1). In addition, butylamine, which is a partial structure of lysine, was easily transformed into butylcarbamic acid in $scCO_2$ in quantitative yield, and then it reverted to butylamine in air within 24 h. In supercritical ethane instead of $scCO_2$ only 5% enantiomeric excess was observed (entry 2). From these results we found the following: (i) Enantioselectivity was observed only in $scCO_2$. (ii) Formation of carbamic acid from an amine was a reversible reaction. (iii) Being unable to form carbamic acid from an amino group, the reaction media could not improve the enantioselectivity. These considerations supported our proposed mechanism of enantioselectivity.

Though the actual reasons for the effect of $scCO_2$ may be the focus of debate, there is no doubt that $scCO_2$ has potential capability to develop the activity as well as the enantioselectivity, even if no enantioselectivity is observed in conventional organic media. We are currently investigating the generality, limitation and details of the mechanism of enantioselectivity. Results will be reported in due course.

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Table 5. CHIRAZYME® L-2-catalyzed asymmetrization of the diacetate **1**

Entry	Media	Conversion (%)	**2** yield (%)	ee (%)	**3** yield (%)
1[a]	Hexane	72	61	0	11
2[b]	scC_2H_6	51	35	5	16

[a] Reaction was carried out at 40°C for 24 h in the presence of methanol (2 equiv.) and CHIRAZYME® L-2 (1 g equiv.) which was exposed to $scCO_2$ for 24 h before use.
[b] Reaction was carried out in a similar manner as in $scCO_2$.

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