Rate Enhancement of Lipase-catalyzed Reaction in Supercritical Carbon Dioxide

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Lipase-catalyzed kinetic resolution of various 1-arylethanols was performed in supercritical carbon dioxide ($scCO_2$), in hexane and without solvent. The use of $scCO_2$ enhances reactivity especially when a cross-linked enzyme aggregate (CLEA) was used.

Supercritical carbon dioxide ($scCO_2$) has been used as a solvent for organic synthesis due to its environmental friendliness, high diffusivity that increases the rate of diffusion-limited reactions, ease of product separation from solvents, etc.[1] As a catalyst for reactions, chemical catalysts have been widely used.[1] Although not as extensively as chemical catalysts, biocatalysts have also been used due to high chemo-, regio-, and enantioselectivities and high reactivity.[2,3]

Among biocatalytic reactions in $scCO_2$, lipase-catalyzed kinetic resolution is one of the most important reactions because asymmetric synthesis is possible. Therefore, in this study, detailed examinations of substrate specificity for kinetic resolution by *Candida antarctica* lipase B, especially using 1-arylethanols, were conducted using a batch $scCO_2$ system because many 1-arylethanols have been used as intermediates for the synthesis of pharmaceuticals, agrochemicals, and natural products.[4] The reaction proceeded with very high enantioselectivity, and moreover, it was found that reactivity was improved by using $scCO_2$ over the cases using hexane[5] and without solvent. Interestingly, when two different methods of enzyme preparation (Novozym and CLEA), derived from the same enzyme (*Candida antarctica* lipase B), were compared, rate enhancement was more obvious when CLEA was used.

Using a lipase from *Candida antarctica* as a catalyst and vinyl acetate **2** as an acetyl donor, kinetic resolution of 1-phenylethanol derivatives **1** was conducted in $scCO_2$[6] or in hexane as well as without solvent. As shown in Figure 1, (*R*)-**1** selectively reacted, resulting in the formation of (*R*)-**3** and unreacted (*S*)-**1**. Absolute configurations were determined by comparing the GC retention times with the authentic samples prepared by the methods reported in the literature.[4a,7]

The results using an immobilized lipase preparation, Novozym, are listed in Table 1. The conversions of $scCO_2$ reactions under pressure of 9 MPa were higher than in hexane or under non-solvent conditions. The rates of the reactions at 13 MPa were similar to those at 9 MPa (data not shown). The effect of the position of the substituents on the phenyl ring was obvious. The conversion was in the order of: **1b** (*p*-Br), **1c** (*p*-CH_3), **1d** (*p*-CF_3) > **1a** (H) > **1e** (*m*-CF_3) > **1f** (*m*,*m*-$(CF_3)_2$). When there is a substituent at the *para* position, conversion was better than unsubstituted substrate **1a**, regardless of the electron donating or withdrawing ability of the substituents.

The enantioselectivity evaluated by *E*-value[8] was extremely high ($E > 1000$) for the reaction in $scCO_2$ or hexane except for the case of **1c**. However, when the solvent was not used, enantioselectivity decreased for cases **1d** and **1f**.

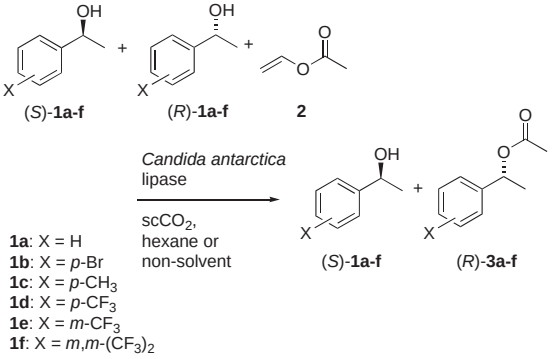

Figure 1. Kinetic resolution of 1-arylethanols by lipase.

Next, different preparations of the same lipase from *Candida antarctica*, CLEA, were used for the kinetic resolution of **1a** in $scCO_2$ or in hexane as well as without solvent. CLEA 103 is the lipase preparation suitable for transformations in aqueous solvents (hydrolysis), and CLEA 301 is developed for transformation in organic media (transesterification).[9] For the reaction in $scCO_2$, both preparations almost equally catalyzed the reaction with high efficiency. The conversion was higher than those using Novozym when the same enzyme weight was used. The intriguing point is that the rate acceleration of the CLEA catalyzed reaction using $scCO_2$ is more prominent than cases using Novozym. When Novozym was used, conversion improved from 25% (hexane or non-solvent) to 31% ($scCO_2$) (Table 1); but when CLEA was used, it improved from 13–35% (hexane or non-solvent) to over 46% ($scCO_2$) (Table 2).

Although the reasons for the rate enhancement using $scCO_2$ for Novozym and CLEA catalyzed reactions remain unclear at present, plausible causes may be related to three factors: 1) desolvation of enzyme and/or substrate as reported to cause rate acceleration for homogeneous reactions using lipid-coated enzymes,[2a,2b] 2) conformational changes of the enzyme by adsorption, absorption, and/or binding of CO_2 to the enzyme partially through formation of carbamates from CO_2 and the free amine groups on the surface of the enzyme as reported previously,[2d,3c,3d,10] or 3) the high diffusivity of $scCO_2$.[11] Because rate acceleration using CLEA (enzyme aggregate) was larger than that using Novozym, high diffusivity of $scCO_2$ may be more impor-

Table 1. Novozym catalyzed kinetic resolution of **1** in various solvents

Substrate/Conditions		(*S*)-**1**[a] ee/%	(*R*)-**3** ee/%	Conv.[b] /%	*E*
1a	$scCO_2$ 9 MPa	45.8	99.9	31	>1000
1a	Hexane	32.9	>99.9	25	>1000
1a	Non-solvent	32.9	>99.9	25	>1000
1b	$scCO_2$ 9 MPa	90.2	99.8	48	>1000
1b	Hexane	75.3	>99.9	43	>1000
1b	Non-solvent	56.0	>99.9	36	>1000
1c	$scCO_2$ 9 MPa	80.4	96.1	46	125
1c	Hexane	57.0	98.5	37	230
1c	Non-solvent	57.1	97.9	37	167
1d	$scCO_2$ 9 MPa	65.3	99.6	40	>1000
1d	Hexane	52.6	>99.9	34	>1000
1d	Non-solvent	18.9	97.4	16	92
1e	$scCO_2$ 9 MPa	30.6	>99.9	24	>1000
1e	Hexane	7.4	>99.9	7	>1000
1e	Non-solvent	3.8	>99.9	4	>1000
1f	$scCO_2$ 9 MPa	23.2	>99.9	19	>1000
1f	Hexane	10.5	>99.9	10	>1000
1f	Non-solvent	10.8	98.8	10	154

Conditions: 40 °C, 2 h, Enzyme: 5.0 mg, substrate: 100 mg, vinyl acetate: 0.50 mL, solvents ($scCO_2$ or hexane): 10 mL. [a]Recovered alcohols. [b]Conversion to **3** based on the starting amount of **1**. No detectable side reaction occurred. Higher conversions were obtained with longer reaction times.

Table 2. CLEA (cross-linked enzyme aggregate) catalyzed kinetic resolution of **1a** in various solvents

Enzyme	Conditions	(*S*)-**1a**[a] ee/%	(*R*)-**3a** ee/%	Conv.[b] /%	*E*
CLEA103	$scCO_2$ 9 MPa	85.5	99.6	46	>1000
CLEA103	Hexane	15.4	>99.9	13	>1000
CLEA103	Non-solvent	34.2	99.5	26	540
CLEA301	$scCO_2$ 9 MPa	93.8	>99.9	48	>1000
CLEA301	Hexane	27.3	>99.9	21	>1000
CLEA301	Non-solvent	52.8	99.5	35	624

Conditions are shown in Table 1. [a]Recovered alcohol. [b]Conversion to **3a** based on the starting amount of **1a**. No detectable side reaction occurred.

tant for reactions by CLEA than that by Novozym; for CLEA reactions, the enzyme inside the aggregate probably catalyzed the reaction more efficiently in $scCO_2$ than in hexane or under non-solvent conditions due to the high diffusivity of $scCO_2$. The degree of rate enhancement is also different between the substrates, so the reasons are probably rather complex. The development of methods to understand the mechanism are in progress in our laboratory.

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5 Lipase-catalyzed reaction in hexane is usually faster than that in other hydrophilic solvents.

6 Visual inspection of the reaction mixture in an autoclave equipped with sapphire windows showed that the reactants and products were all soluble in $scCO_2$ under the reaction conditions at 9 and 13 MPa.

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