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Pressurized Carbon Dioxide in Biocatalysis: Enhanced Catalytic Activity of Lipase

Hoang Nam Hai

[Introduction]

The scope of biocatalysis, particularly in enantioselective synthesis of target molecules for pharmaceuticals, has been extended through the use of enzymes in organic solvents and in "green" nonaqueous media. Carbon dioxide (CO_2), which has numerous positive impacts on green chemistry, has been utilized as novel and sustainable reaction platform for lipase catalysis in this study (Figure 1).

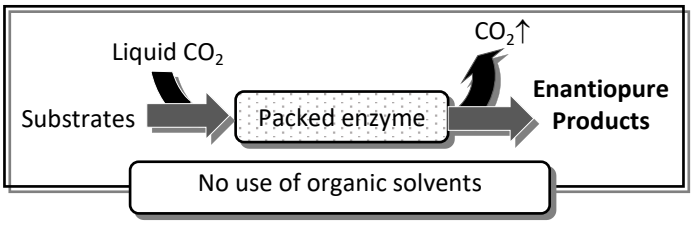

Fig. 1 Biocatalytic synthesis using liquid CO_2

[Results and Discussion]

1. Liquid carbon dioxide: Broadening substrate scope of lipase catalysis

The kinetic resolution of typical substrate *rac*-1-phenylethanol by *Candida antarctica* lipase B (CALB, Novozym 435) using vinyl acetate as an acyl donor was investigated as a model reaction in liquid CO_2 and compared with that in conventional organic solvents. The enzyme exhibits the highest activity in liquid CO_2 with excellent enantioselectivity. The potential applicability of liquid CO_2 fluid for large-scale enantioselective synthesis was also examined with continuous flow bioreactors. The continuous kinetic resolution of *rac*-1-phenylethanol by the immobilized lipase in a packed-column reactor stably afforded corresponding enantiopure products with minimum waste generated (Figure 1). Interestingly, by using in liquid CO_2. The substrate scope CALB was found to be expanded towards sterically bulky *sec*-alcohols such as 1-phenyldodecanol, of which the lipase has been known to have no reactivity in standard conventional solvent.

2. CO_2-expanded bio-based liquids: Modulating lipase activity by controlling physicochemical properties of solvent

As emerging alternative solvents, bio-based liquids provide environmentally advantages, such as sustainable production, better biodegradability and lower toxicity. However, some bio-based liquids with high polarity are not favourable solvents for biocatalysis, such as 2-methyltetrahydrofuran (MeTHF), as it often witnessed in decreased lipase activity. With tunability, pressurized CO_2 was employed to manipulate the physicochemical properties

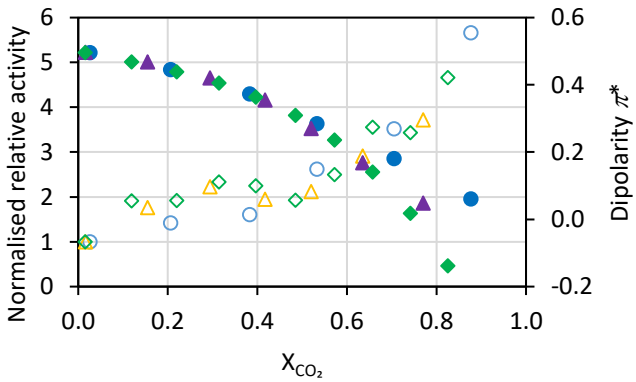

Fig. 2 Tuning CALB activity in CO_2-expanded MeTHF (unfilled legends) and solvent property (dipolarity π^*, filled legends) as a function of CO_2 mole fraction X_{CO_2}. Reaction condtions: 0.10 mmol *rac*-1-adamantylethanol, 0.53 mmol vinyl acetate, 10 mg Novozym 435, 1.0 mL MeTHF, pressures ranging from 0.1 MPa to 9 MPa. Reaction rate (μmol substrate/mg enzyme/h) was normalized to 1 for the reaction at X_{CO_2}=0 of each temperature (round 20 °C, triangle 40 °C, diamond 60 °C) .

such as dipolarity π^* of many bio-based liquids into favorable platforms for lipase catalysis (herein called as CO_2-expanded bio-based liquids, Figure 2).

3. Molecular behaviour of *Candida antarctica* lipase B in pressurized carbon dioxide

The underlying mechanism of crucial role of CO_2 to induce increased activity of lipase was studied. Molecular dynamics simulation of CALB showed that pressurized CO_2 can modify the structure flexibility of the active-site entrance, which allows bulkier substrates to reach the active site cavity of CALB. Moreover, the volume of main cavity of CALB was found to be enlarged in pressurized CO_2, so that the enzyme can comfortably accommodate bulkier substrates. On the basis of the experimental and computational findings, the structure of CALB in pressurized CO_2 were proposed as in Figure 3.

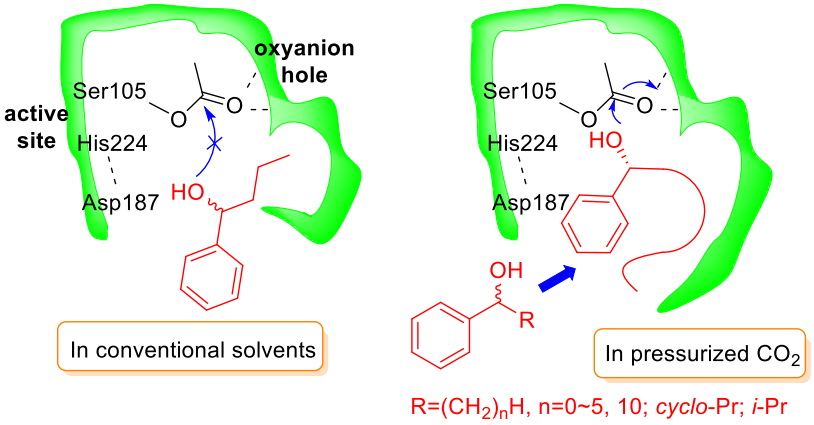

Fig. 3 Proposed model of CALB structures

[Conclusion]

Herein, pressurized CO_2 (liquid CO_2 and CO_2-expanded bio-based liquids) was proved as superior media for lipase catalysis. In pressurized CO_2 not only much higher activity were possible, also CALB retained its originally high enantioselectivity towards various sterically bulky *sec*-alcohols. The molecular dynamics simulation of CALB in pressurized CO_2 showed important changes in the lipase conformation that the lipase became more tolerant to bulkier substrates. With these positive findings, I expected that pressurized CO_2 is promising non-aqueous media for other biocatalysis.

[Publications]

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