Effective biocatalytic transgalactosylation in a supercritical fluid using a lipid-coated enzyme

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A lipid-coated β-D-galactosidase is soluble and acts as an efficient trangalactosylation catalyst (the reverse hydrolysis reaction) in supercritical carbon dioxide ($scCO_2$).

Supercritical fluids (scFs) have become attractive as media for chemical reactions, as well as for extraction and chromatography, in the last decade;[1] their physical properties can be manipulated by small changes in pressure or temperature, and several of these properties (*e.g.* density, diffusion and viscosity) are intermediate between those of gases and liquids.[2] The larger diffusion rate in a scF compared to a liquid can be expected to increase the reaction rate.

Several organic reactions in scF have been achieved using homogeneously soluble organometallic complexes as catalysts.[3] Application of scFs for enzymatic reactions has also been reported using immobilized enzymes, as well as native enzymes.[4] However, since the immobilized and native enzymes are not soluble or particularly not stable in scFs, results comparable to those found in aqueous or organic solvents have not been obtained.[4] We have reported that the lipid-coated enzymes such as lipases,[5] phospholipases[6] and glycosidases[7] are soluble in most organic solvents and can catalyze reverse hydrolysis reactions such as esterification and transglycosylation in homogeneous organic media. We expected that lipid-coated enzymes would be homogeneously soluble in scFs as well as organic media, and would show efficient catalytic activity due to the high diffusion rates and the low viscosity found in scFs.

Here we report the high catalytic activity of transgalactosylation using a lipid-coated β-D-galactosidase in supercritical carbon dioxide ($scCO_2$). The reasons carbon dioxide was chosen as the scF are as follows: (i) CO_2 becomes a scF above 31.0 °C and 73.8 atm, conditions which are easily accomplished with gentle heating from ambient temperature and a commercial liquid chromatography pump, (ii) the solvent properties of $scCO_2$ can be continuously varied by changing the pressure or temperature, and (iii) CO_2 is non-toxic and the medium is easily removed by decompression to atmospheric pressure.

A lipid-coated β-D-galactosidase (from *Bacillus circulans*) was prepared by mixing aqueous solutions of enzyme and lipid molecules in the same way as reported previously.[5–7] It was confirmed from elemental analysis, UV absorption, and gel chromatography in CH_2Cl_2 that one enzyme is covered by about 200 ± 50 lipid molecules as a monolayer and that the protein content in the complex is 7 ± 1 wt%.[5–7] The lipid-coated enzyme was also found to be soluble (*ca.* 0.1 mg ml^{-1}) in $scCO_2$ in the range of 32–60 °C and 74–200 atm, by the observation using a pressure-resistant glass vessel (Taiatsu Techno, Co., Tokyo, volume: 10 ml), but not very soluble in liquid CO_2 (at 20 °C and 100 atm) and insoluble in gaseous CO_2 (at 40 °C and 40 atm).

Transgalactosylation reactions were carried out as follows. In a stainless steel or pressure-resistant glass vessel, both the substrates (1-*O*-*p*-nitrophenyl-β-D-galactopyranoside and 5-phenylpentan-1-ol) and a lipid-coated β-D-galactosidase were added, then liquid CO_2 was injected at 100–150 atm using a LC pump (Jasco PU-980 HPLC pump) connected to a CO_2 gas cylinder. The vessel was warmed with magnetic stirring above 40 °C to create the supercritical state, and the pressure was kept constant (±0.1 atm) by a back pressure regulator (JASCO 880–81). At the appointed time, the vessel was degassed carefully under cooling at 0 °C. The residual powder was solubilized in MeCN and analyzed by a HPLC.

Fig. 1 shows typical time courses of the transgalactosylation from 1-*O*-*p*-nitrophenyl-β-D-galactopyranoside (0.1 mM) to 5-phenylpentan-1-ol (1.0 mM) catalyzed by a lipid-coated β-D-galactosidase at 40 °C both in $scCO_2$ at 150 atm and in isopropyl ether at atmospheric pressure. In $scCO_2$, 1-*O*-(5-phenylpentyl)-β-D-galactopyranoside was obtained as the only transgalactosylated product in 72% yield after 3 h, at which point the reaction reached equilibrium. We have reported that the lipid-coated β-D-galactosidase can catalyze the same transgalactosylation in conventional organic solvents such as isopropyl ether.[7] The transgalactosylation in $scCO_2$ was 15-fold faster than in isopropyl ether. In this case, both the enzyme and the substrate

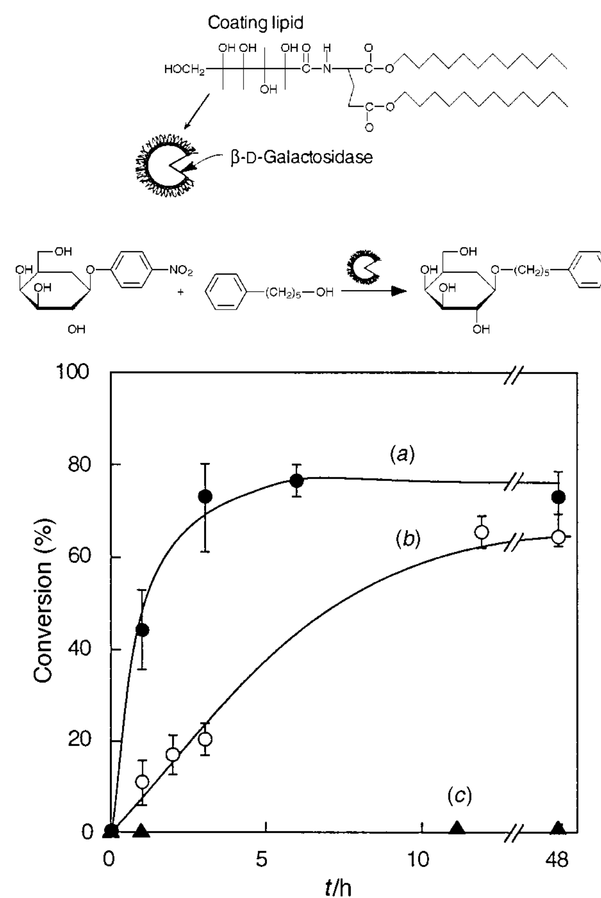

Fig. 1 A schematic illustration of a lipid-coated β-D-galactosidase and time-courses of transgalactosylation from 1-*O*-*p*-nitrophenyl-β-D-galactopyranoside (0.1 mM) to 5-phenylpentan-1-ol (1 mM) at 40 °C catalyzed by β-D-galactosidase (1 mg of protein) in 10 ml: (*a*) a lipid-coated enzyme in $scCO_2$ with 150 atm, (*b*) a lipid-coated enzyme in isopropyl ether, and (*c*) a native enzyme in $scCO_2$ with 150 atm

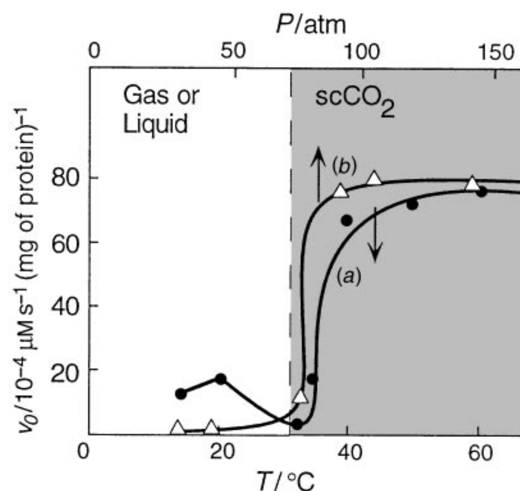

Fig. 2 Effect of (*a*) changing temperature at 150 atm and (*b*) changing pressure at 40 °C on the initial rate of transgalactosylation

were soluble in $scCO_2$, so the increase in the rate may be due to a decrease in the degree of solvation of the substrate. Transgalactosylations also occurred rapidly with acceptor alcohols having large alkyl groups, such as 1,2-*O*-bis(dodecyl)glycerol (conversion 45%) and cholesterol (conversion 15%).

When a lipid-coated lipase (from *Rhizopus delemar*) was prepared and applied as an esterification catalyst for monolaurin and lauric acid, the esterification rate increased 10-fold in $scCO_2$ over that in atmospheric isooctane (data not shown).[8] Although several studies on enzyme reactions in $scCO_2$ using native or immobilized enzymes have been performed, an improvement of reactivity over the reaction in atmospheric liquid media has not been reported.[4] Actually, the native β-D-galactosidase was observed to hardly catalyze the transglycosylation in $scCO_2$, as shown in Fig. 1(*c*). This is due to the insolubility and instability of native enzymes in $scCO_2$.

FT-IR spectra usually provide information about secondary structures, such as the content of α-helix and β-sheet domains in proteins.[9] FT-IR spectra were taken of the lipid-coated β-D-galactosidase in $scCO_2$[10] and a native β-D-galactosidase in D_2O solution. It was indicated from the peak strength of the amide I band at 1600–1700 cm^{-1} that the content of α-helix (21%) and β-sheet (26%) structures of the lipid-coated enzyme in $scCO_2$ agreed reasonably well with those (α-helix: 19%; β-sheet: 30%) of a native enzyme in D_2O solution. Thus, the structure of the lipid-coated enzyme in $scCO_2$ is not significantly changed from that in aqueous buffer solution.

One of the advantages of scFs as reaction media is that their physicochemical properties, such as diffusiion rate, density, polarity and viscosity, can be continuously changed by varying the temperature or pressure of the $scCO_2$ state.[2] The effects of changing temperature and pressure on the initial rates of the transgalactosylation catalyzed by a lipid-coated enzyme are shown in Fig. 2.[11] When the temperature was changed at a constant pressure of 150 atm [Fig. 2(*a*)], galactosylations were very slow below 31 °C, where the medium exists as liquid CO_2 and the lipid-coated enzyme is barely solubilized. The reactivity increased above 31 °C, where the lipid-coated enzyme is solubilized. Since the enzyme activity in aqueous buffer solution increased gradually with increasing temperature from 20 to 60 °C, this activity change depending on temperature is explained by the special physical property changes of $scCO_2$. A similar tendency was observed when the pressure was changed from 50 to 150 atm at 40 °C [Fig. 2(*b*)]. Gaseous CO_2 could not solubilize the lipid-coated enzyme below 72.9 atm (the critical pressure) and the enzyme activity drastically increased in $scCO_2$ depending on the pressure. When the temperature and pressure were changed repeatedly in the region in Fig. 2, the enzyme activity could be controlled reversibly over at least 10 cycles.

In conclusion, lipid-coated enzymes such as β-D-galactosidase and lipase are soluble and can catalyze transgalactosylation and esterification in supercritical CO_2. The enzyme activity was 10–15 times larger than that in conventional organic media. We could switch the enzyme activity on and off by adjusting the pressure or temperature of the CO_2 media across or within the $scCO_2$. In addition, compared with organic solvents $scCO_2$ is non-toxic and easily removed by decompression to atmospheric pressure, and thus is suitable for the biotransformation of food products and drugs. We believe that the combination of a lipid-coated enzyme and a $scCO_2$ reaction medium will form a versatile new system for biotransformation studies.

Notes and References

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