

Reversible Control of Enzymatic Transglycosylations in Supercritical Fluoroform Using a Lipid-Coated β -D-Galactosidase

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We have reported that lipid-coated hydrolytic enzymes, in which hydrophilic headgroups of lipids interact with the enzyme surface and lipophilic two long alkyl chains extend away from its surface that solubilize the enzyme in organic solvents, act as an efficient catalyst for the reverse hydrolysis reactions such as esterification by lipase and transglycosylation by glycosidase.¹ The catalytic activities of the lipid-coated enzymes were 2–100 times higher than that of other systems, including enzymes in organic/aqueous emulsion,² dispersed powdered enzymes,³ and poly(ethylene glycol)-grafted lipase in organic solvents.⁴ However, the activity and stability of the lipid-coated enzymes in organic media largely depend on their polarity, as well as other enzyme systems. Thus, the esterification rate of the lipid-coated lipase was high in nonpolar isooctane or benzene, but denatured in the polar media such as chloroform and tetrahydrofuran.¹ This is a large disadvantage of enzyme reactions in organic media, because the polar solvents are attractive for the high reactivity and high solubility of substrates.

Recently, supercritical fluids (scFs) became interesting as a new media for chemical reactions including enzymatic reactions, since their physical properties (e.g., polarity, diffusion, and viscosity) are intermediate between those of gases and liquids, and they can be tuned by small changes in pressure or temperature.^{5–7} We have reported that the lipid-coated enzymes are soluble and show high activity in supercritical carbon dioxide (scCO₂) as well as organic media.⁷

In this communication, we report that the transgalactosylation rate catalyzed by the lipid-coated β -D-galactosidase in supercritical fluoroform (scCHF₃) can be reversibly controlled by changing temperature or pressure (reflecting polarity changes) without damaging enzymes. The reasons scCHF₃ was chosen instead of scCO₂ are that the high solubility of scCHF₃ for both the lipid-coated enzyme and substrates and the dielectric constants (ϵ) of scCHF₃ can be changed from 1 to 7 (corresponding to the ϵ value of hexane to tetrahydrofuran as organic media) by manipulating either the temperature or the pressure of scFs.^{5,7}

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, in which the protein content was 7 ± 1 wt %.^{1,7} The lipid-coated enzyme was freely soluble (1–5 mg in 10 mL) in scCHF₃ in the range of 30–60 °C and 5–25 MPa.

Transgalactosylation reactions were carried out as follows.^{1c,7b} In a stainless steel vessel with pressure-resistant glasses both substrates of 1-*O*-*p*-nitrophenyl- β -D-galactopyranoside and 5-phenyl-1-pentanol and a lipid-coated β -D-galactosidase were added, then liquid CHF₃ was injected at 5–25 MPa from a LC pump (Jasco PU-980 HPLC pump) connected to a CHF₃ gas cylinder. The vessel was warmed with stirring magnetically above 25 °C to create a supercritical state, and the pressure was kept constant (± 0.1 MPa) by a backpressure regulator. At every appointed time, the vessel

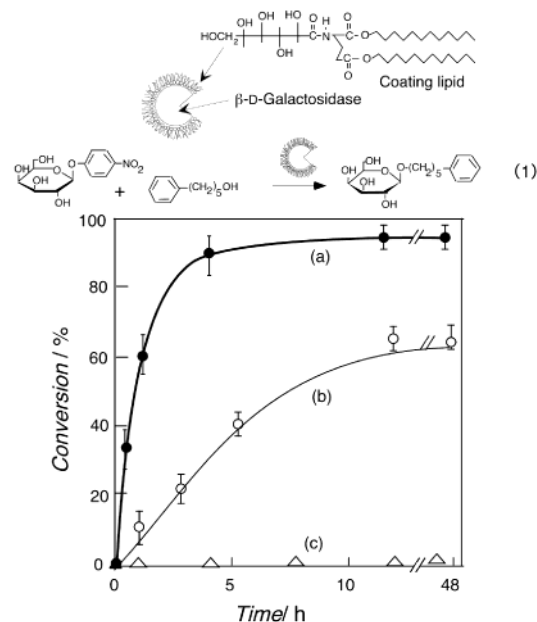


Figure 1. A schematic illustration of a lipid-coated β -D-galactosidase and time courses of transgalactosylation from 1-*O*-*p*-nitrophenyl- β -D-galactopyranoside (10 mM) to 5-phenyl-1-pentanol (100 mM) at 37 °C catalyzed by β -D-galactosidase (1 mg of protein) in 10 mL: (a) a lipid-coated enzyme in scCHF₃ with 6 MPa, (b) a lipid-coated enzyme in atmospheric diisopropyl ether, and (c) a native enzyme in scCHF₃ with 6 MPa.

was degassed carefully under cooling at 0 °C. The residual powder was solubilized in CH₃CN and analyzed by HPLC.

Figure 1 shows typical time courses of the transgalactosylation from 1-*O*-*p*-nitrophenyl- β -D-galactopyranoside to 5-phenyl-1-pentanol catalyzed by a lipid-coated β -D-galactosidase at 37 °C both in scCHF₃ at 6 MPa and in the atmospheric diisopropyl ether. In scCHF₃, 1-*O*-(5-phenylpentyl)- β -D-galactopyranoside was obtained as the only transgalactosylated product in 90–95% yield after 5 h. The transgalactosylation in scCHF₃ was 20-fold faster than that in atmospheric diisopropyl ether. The rate increase in scCHF₃ may be due to the decrease of viscosity or the degree of solvation to substrates, as compared with the conventional solvents. When the native β -D-galactosidase was employed in scCHF₃ instead of the lipid-coated one, the reaction hardly proceeded because the native enzymes were insoluble and existed as powders (curve c, Figure 1). Several enzymatic reactions using immobilized enzymes or native enzymes in scFs have been reported; however, they are not soluble in scFs and results comparable to this study have not been obtained.⁶

One of the future uses of scF as a reaction media is a result of physicochemical properties such as the dielectric constant (ϵ) of the media that can be tuned by continuous changes of temperature or pressure in the scF state.^{5,8} The effects of continuous changes

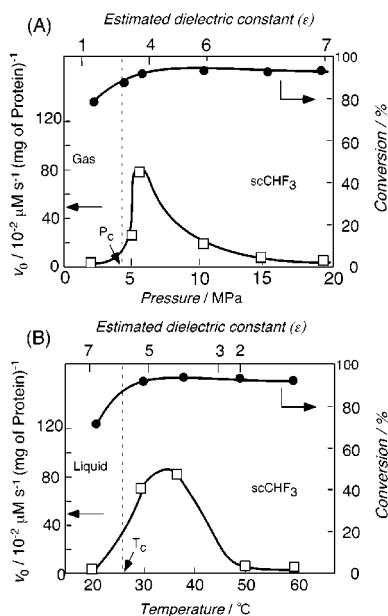


Figure 2. Effect of (A) pressure changes at 37 °C and (B) temperature changes with 6 MPa on the initial rates (□) and the conversion (●) of transgalactosylation catalyzed by the lipid-coated β -D-galactosidase in scCHF₃.

of temperature or pressure on both the initial rates and conversion of the transgalactosylation catalyzed by a lipid-coated enzyme are shown in Figure 2. When pressures were continuously changed at a constant temperature of 37 °C (Figure 2A), galactosylations were very slow below $P_c = 4.8$ MPa, where the medium exists as gaseous CHF₃ and the lipid-coated enzyme exist as powders. Reaction rates were drastically increased above 4.8 MPa and then decreased drastically above 10 MPa, where the lipid-coated enzyme is homogeneously solubilized in scCHF₃. However, the conversions were constant at be 95% in the 6–20 MPa range. A similar tendency was observed when temperatures were changed from 20 to 60 °C at a constant pressure of 6 MPa; the initial rate showed a bell shape behavior, although the conversion was constantly high as 80–95% (Figure 2B). The enzyme activity in liquid CHF₃ at 20 °C with 6 MPa was similar to that in the conventional polar organic solvents. Since the β -D-galactosidase activity in aqueous buffer solution increased gradually with increasing temperatures from 20 to 60 °C, these activity changes are explained by special physical property changes of scCHF₃. When the temperature or pressure of scCHF₃ is changed continuously, it is well-known that dielectric constants (ϵ) of the media changed continuously,⁵ and these estimated apparent ϵ values are shown at the upper x-axis of Figure 2.

In Figure 3A, the initial rate and conversion are plotted against apparent ϵ values in different pressures (3–20 MPa) and temperatures (20–60 °C) of scCHF₃ shown in Figure 2. The initial rates showed a bell-shaped curve and the conversion was constantly high independent of ϵ values of scCHF₃. When the lipid-coated β -D-galactosidase was employed in various organic solvents as a catalyst for the same transglycosylation in eq 1 of Figure 1, both the initial rate and conversion were drastically decreased in the polar solvents of chloroform, ethyl acetate, and tetrahydrofuran (Figure 3B).^{1c} A similar denature of enzymes in the polar organic solvents has been shown in the esterification catalyzed by a lipid-coated lipase.^{1b} Thus, the enzyme is active in the non-polar solvents such as isooctane, benzene, and diisopropyl ether, however, it easily denatured in the polar solvent and did not revert the reactivity. When scCHF₃ was employed as the medium, the polarity could be tuned by changing

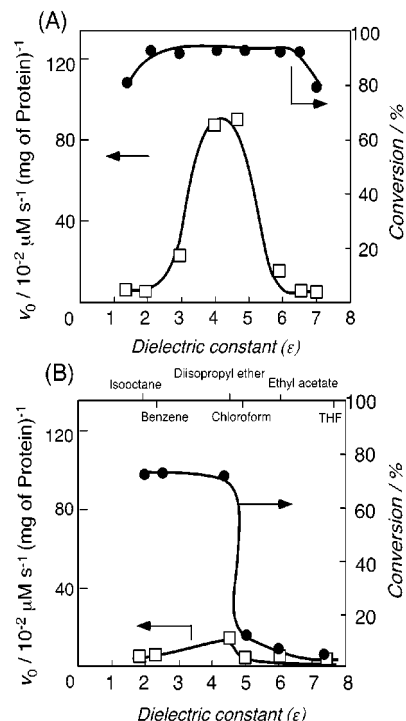


Figure 3. The initial rates (□) and conversions (●) of transgalactosylations were plotted against dielectric constants (ϵ) (A) in scCHF₃ obtained by pressure and temperature changes and (B) in atmospheric organic solvents by changing media.

continuously pressure or temperature without changing media, and the reaction rate could be controlled reversibly by keeping the high conversion. Thus, the enzyme can be kept inactive at 20 °C with 6 MPa or 50 °C with 20 MPa, and reverted to the active form at 30 °C with 6 MPa in scCHF₃. This could be continued reversibly at least 10 cycles.

In conclusion, the lipid-coated β -D-galactosidase is soluble and can catalyze transgalactosylation 2–20 times faster in supercritical CHF₃ than in the conventional organic media. The reaction rate could be switched on and off by adjusting the pressure or the temperature of scCHF₃. Since CHF₃ has no chloride or bromide atoms, it is not included in ozone-depletion fluorocarbons and can be practically used by recovering and recycling. We believe that the combination of the lipid-coated enzyme and scCHF₃ reaction media will become a new system for biotransformation studies.

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