Biocatalytic One-step Synthesis of *n*-Octyl β-D-Xylotrioside and Xylobioside from Xylan and *n*-Octanol in Supercritical Carbon Dioxide

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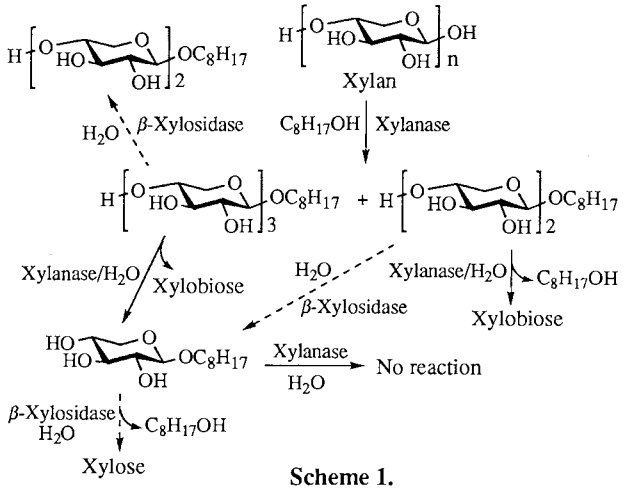
n-Octyl β-D-xylotrioside and xylobioside, which are effective next generation surfactants, can be prepared by the one-step reaction of xylan and *n*-octanol using acetone powder (acetone-dried cells) of *Aureobasidium pullulans* KK415 (ATCC 201145) as the enzyme source of xylanase in supercritical carbon dioxide ($scCO_2$). Yields of the octyl glycosides were significantly increased using $scCO_2$. Furthermore, without $scCO_2$, *n*-octyl xylotrioside was hardly produced.

In recent years, the recognition and establishment of a new field of "green chemistry" have been realized as a necessary goal for the sustainable development in the next century. Some of the challenges for the greening of chemistry in the field of surfactants may include the application of biocatalysts and the use of naturally abundant and recyclable carbohydrates. Among them, xylan is one of the most abundant carbohydrates, occurring as the main component of hemicellulose. It is reported that alkyl β-D-xylobioside and β-D-xylotrioside exhibited excellent surface activities, such as stable foam forming, when compared to those of the monomeric alkyl β-D-xyloside.[1] However, the preparation of alkyl β-D-xylobioside and β-D-xylotrioside requires a multistep reaction as well as anhydrous conditions, and also the selective production of β-anomers may not be industrially feasible. Based on these facts, the enzymatic method may be the best way to prepare the alkyl β-D-oligoxyloside. We previously reported that the preparation of alkyl β-D-xylobioside and xyloside using a biocatalyst; however, no alkyl β-D-xylotrioside was accumulated in the reaction mixture, ascribed to the rapid hydrolysis of xylotrioside by xylanase.[2,3] Reaction schemes of the transglycosylation reaction of xylan and octanol with respect to xylanase and β-xylosidase of *A. pullulans* are shown in Scheme 1. The xylanase could catalyze the transglycosylation reaction of xylan and octanol to produce octyl xylotrioside and xylobioside, and the former was quickly hydrolyzed by the same xylanase into octyl xyloside and xylobiose as the final products. The latter was also hydrolyzed by xylanase to liberate xylobiose and octanol as the final product.[4] At the same time, octyl xylotrioside, xylobioside and xyloside were slowly hydrolyzed by β-xylosidase to liberate xylose. This β-xylosidase showed no transglycosylation activity under these conditions.

It is known that the solvent properties of supercritical carbon dioxide ($scCO_2$) can be continuously varied by changing the pressure and temperature. The activities of xylanase may be controlled by changing the pressure and temperature of the $scCO_2$. It was recently revealed that the activity of lipid-coated lipase could be reversibly controlled by changing the pressure and temperature of the $scCO_2$.[5]

In this paper, the one-step synthesis of alkyl β-D-oligoxylosides including xylotrioside was studied in $scCO_2$ in order to regulate the activities of xylanase which was responsible for the direct transglycosylation of xylan and alcohol. As the biocatalyst for the reaction, acetone-dried resting cells (acetone powder) of *A. pullulans* were used for the production of the alkyl β-D-oligoxylosides. The enzymatic synthesis of the alkyl xyloside has been reported;[6-9] however, the formation of xylotrioside and the transglycosylation reaction in $scCO_2$ were not reported.



Scheme 1.

The acetone powder of *Aureobasidium pullulans* KK415 (ATCC 201145) was prepared according to a previous paper.[3] The xylanase and β-xylosidase activities of the acetone powder were 3.5 mU/mg for xylan and 1.9 mU/mg for *p*-nitrophenyl β-D-xyloside, respectively.[10] Preparation of the *n*-octyl β-D-oligoxylosides was carried out by the transglycosylation reaction of xylan[11] and *n*-octanol using the acetone powder in $scCO_2$. A typical preparation procedure is as follows. A mixture of 20 mg xylan, 10 mg acetone powder, 0.9 mL *n*-octanol and 0.1 mL of 0.1 mol/L acetate buffer (pH 4.0) was placed in a 10 mL stainless steel vessel along with a magnetic stirring bar, and $scCO_2$ was injected at 150 kg/cm^2 from a CO_2 pump (JASCO Corp., Ltd). The reaction vessel was warmed to 65 °C with stirring for 16 h at a constant pressure using a back pressure regulator (JASCO Corp., Ltd). After the reaction, the reaction vessel was cooled using dry ice-methanol and slowly degassed. Ten milliliters of ethanol was added to the residue and the insoluble xylan and acetone powder were removed by filtration. After evaporation of the ethanol, the yields of the *n*-octyl xylotrioside, xylobioside and xyloside in the incubation mixture were directly analyzed by HPLC using authentic standards.[12] The structures of the transglycosylation products were analyzed by HPLC, IR, MALDI-TOF MS, ^{1}H NMR and ^{13}C NMR spectroscopies.[1,2]

It was found that the transglycosylation of xylan and *n*-octanol by xylanase of the acetone powder of *A. pullulans* in $scCO_2$ produced *n*-octyl β-D-xylotrioside, xylobioside and xyloside. Table 1 summarizes the transglycosylation reaction with/without $scCO_2$. The yields of octyl xylobioside and xyloside were significantly increased by the addition of $scCO_2$ (entries 1

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Table 1. Comaprison of transglycosylation reaction of xylan and *n*-octanol with/without $scCO_2$ at 65 °C for 16 h

Entry	$scCO_2$ / kg cm^{-2}	Octanol / mL	Acetate buffer / mL	Acetone powder / mg	Yield of octyl β-D-oligoxylosides / mg/g xylan		
					n=1	2	3
1[a]	150	0.9	0.1	10	18	52	15
2[b]	---	0.9	0.1	10	8	18	0
3[c]	---	3.6	0.4	2.8	12	47	0

[a]A mixture of 20 mg xylan, *n*-octanol, acetone powder and 0.1 mL of 0.1 mol/L acetate buffer (pH 4.0) in a 10 mL vessel with 150 kg/cm^2 $scCO_2$ was reacted. [b]Reaction conditions were the same as those of entry 1 except that no $scCO_2$ was used. [c]20 mg xylan was used.

and 2). The yields of octyl xylobioside and xyloside were increased by using a large amount of octanol; however, octyl xylotrioside was not accumulated (entry 3). Addition of a small amount of organic solvent, such as 20% acetone, acetonitrile or 1,4-dioxane, significantly decreased the yields of octyl glycosides. It was confirmed that *n*-octyl β-D-xylotrioside was only accumulated when $scCO_2$ was used. Based on these results, it was confirmed that the transglycosylation reaction of xylan and alcohol to produce alkyl xylotrioside and xylobioside was significantly promoted by using the $scCO_2$. The relationships between the concentration of acetone powder and yields of *n*-octyl β-D-xylotrioside, xylobioside and xyloside were analyzed at 65 °C and pH 4.0, and the results are shown in Figure 1. It was found that the yields of *n*-octyl oligoxylosides depended on the concentration of the acetone powder. The highest yields of *n*-octyl xylotrioside and xylobioside were obtained for an acetone powder concentration of 10 mg in a 10 mL $scCO_2$ reaction mixture. On the other hand, the yield of *n*-octyl xyloside gradually increased with increasing concentration of the acetone powder. This is ascribed to the hydrolysis of *n*-octyl xylotrioside and xylobioside by xylanase and β-xylosidase, which were contained in the acetone powder, to produce *n*-octyl xyloside.

The relationship between the reaction temperature and yields of *n*-octyl oligoxylosides was analyzed using a mixture of 20 mg xylan, 10 mg acetone powder, 0.9 mL *n*-octanol and 0.1 mL of 0.1 mol/L acetate buffer (pH 4.0) in a 10 mL vessel with $scCO_2$ at 150 kg/cm^2. These results are shown in Figure 2. It was found that the best reaction temperature, which gave the maximum yields of *n*-octyl xylotrioside, xylobioside and xyloside, was 65 °C. The decrease in the yields of glycosides at the higher temperature of 70 °C may be ascribed to the enzyme stability in $scCO_2$. The reaction pressure of $scCO_2$ was responsible for the yield of the alkyl glycosides and the maximum yields for octyl xylotrioside, xylobioside and xyloside were found at the $scCO_2$ pressure between 90 and 160 kg/cm^2; however, the yields decreased with increasing pressures higher than 160 kg/cm^2. This may be ascribed to the deactivation of the enzyme by the high $scCO_2$ pressure. Such details are now under study.

The time course of the yields of the *n*-octyl glycosides were analyzed at 65 °C. The reaction conditions were the same as those in Figure 2. It was found that the yield of xylobioside quickly increased during the first 16 h, then slightly decreased. Similarly, the yield *n*-octyl xylotrioside increased for the first 6 h, then gradually decreased probably due to the enzymatic

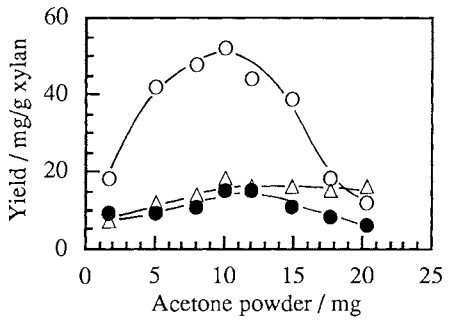

Figure 1. Yields of n-octyl β-D-xylotrioside (●), xylobioside (○) and xyloside (△) by the transglycosylation reaction of 20 mg xylan, 0.9 mL *n*-octanol, acetone powder and 0.1 mL of 0.1 mol/L acetate buffer (pH 4.0) in a 10 mL vessel with 150 kg/cm^2 $scCO_2$ at 65 °C for 16 h.

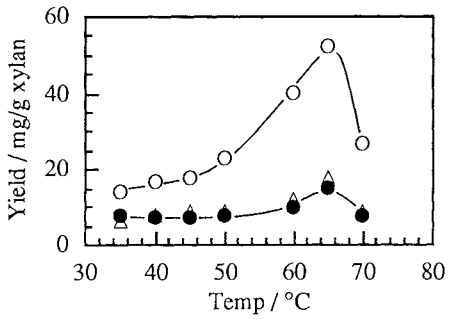

Figure 2. Yields of of n-octyl β-D-xylotrioside (●), xylobioside (○) and xyloside (△) by the transglycosylation reaction of 20 mg xylan, 0.9 mL n-octanol, 10 mg acetone powder and 0.1 mL of 0.1 mol/L acetate buffer (pH 4.0) in a 10 mL vessel with 150 kg/cm^2 $scCO_2$ for 16 h.

hydrolysis of octyl xylotrioside to produce octyl xyloside. On the other hand, the yield of octyl xyloside gradually increased up to 24 h incubation and then remained almost constant.

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11 Xylan from oat spelt was purchased from Fluka Chemie AG.
12 Octyl β-D-oligoxylosides were analyzed using HPLC with a refractive index detector and a commercial HPLC column (Inertosil ODS-2, GL Sciences Inc., Tokyo), with acetonitrile/water (2/1, v/v) as the eluent calibrated with the chemically synthesized authentic standards.