Note

Lipase-Catalyzed Synthesis of Monolauroyl Maltose through Condensation of Maltose and Lauric Acid

Xiaoming Zhang,[1] Takashi Kobayashi,[1] Yoshiyuki Watanabe,[1] Takao Fujii,[1] Shuji Adachi,[1,*] Kazuhiro Nakanishi[2] and Ryuichi Matsuno[1]

[1]*Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*
[2]*Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, 3-1-1 Tsushima-naka, Okayama 700-8530, Japan*

Monolauroyl maltose was synthesized through the immobilized-lipase-catalyzed condensation of maltose and lauric acid in acetone using a batch reactor or a continuous stirred tank reactor. The presence of 4A molecular sieves significantly increased the conversion by the removal of water from the reaction mixture. The surfactant properties of the monolauroyl maltose were measured at different temperatures, and the critical micelle concentration depended little on the temperature.

Keywords: monolauroyl maltose, lipase, condensation, surface tension

Sucrose fatty acid esters synthesized by conventional chemical methods are widely used as detergents and as additives in foods. It has also been reported that the fatty acid esters of maltose and trehalose show antitumor activity (Kohya *et al*., 1986; Okabe *et al*., 1999). Much attention has been paid recently to the enzymatic synthesis of the fatty acid esters of mono- and disaccharides due to its regio- and stereoselectivity (Ducret *et al*., 1996; Woudenberg-van Oosterom *et al*., 1996). Although some reports on the enzymatic preparation of the fatty acid esters of maltose (Ku & Hang, 1995; Woudenberg-van Oosterom *et al*., 1996; Degn *et al*., 1999; 2000; Ferrer *et al*., 2000) have been published, they were performed by a batch reaction on a small scale. In their preparation through the condensation of maltose and a fatty acid, water is produced as a byproduct. The removal of the water by addition of a desiccant, i.e., molecular sieves, can shift the reaction toward the desired product (Ferrer *et al*., 2000). However, a detailed examination of the effect of such an addition on the conversion has not been done for the synthesis of maltose fatty acid esters.

In this context, we first investigated the effects of the molar ratio of a fatty acid, lauric acid, to maltose and the maltose concentration at a fixed molar ratio on the conversion in the absence and presence of 4A molecular sieves for the synthesis of monolauroyl maltose through the condensation catalyzed by *Candida antarctica* lipase in acetone using a small-scale batch reactor. The continuous synthesis of monolauroyl maltose was then carried out using a continuous stirred tank reactor (CSTR), and the surfactant properties of the monolauroyl maltose were also examined.

Materials and Methods

Materials Immobilized lipase from *Candida antarctica*, Chirazyme® L-2 C2, was purchased from Roche Molecular Biochemicals, Mannheim, Germany. Maltose monohydrate, lauric acid, 4A 1/16 molecular sieves, acetone and all other chemicals were purchased from Wako Pure Chemical Industries, Osaka, Japan.

Synthesis of monolauroyl maltose in a batch reaction Prior to use, all the solvents were dehydrated with 4A molecular sieves for at least 24 h. Maltose monohydrate (0.25 to 1.0 mmol), lauric acid (0.25 to 4.0 mmol) and the immobilized lipase (100 mg) were weighed in a glass vial with a screw-cap, and then 5 ml of a solvent was added to the vial. If necessary, the 4A molecular sieves (100 to 500 mg) were put into the vial. The vial was immersed in a water bath at 50°C and vigorously shaken to commence the condensation. The concentrations will, in this paper, be expressed by the moles of substrates or product, or milligram of molecular sieves per liter-solvent. At appropriate intervals, a portion of the reaction mixture was removed and the concentration of the product, monolauroyl maltose, was determined by HPLC with a Nucleosil 5C18 column (4.6×300 mm, Chemco Scientific, Osaka). The eluent used was a mixture of methanol and water (80 : 20, by vol.), and its flow rate was 0.7 ml/min. The eluate was monitored with a Shimadzu RID-6A refractometer (Kyoto). The calibration curve was prepared using the product obtained by the method mentioned below.

Maltose monohydrate (10 mmol), lauric acid (30 mmol) and the immobilized lipase (2 g) were mixed with 200 ml of acetone, and the condensation was carried out at 50°C for 4 days. The reaction mixture was filtered on a sintered glass, and the filtrate was rotary-evaporated. One hundred milliliters of hexane was added to the residue. The solution was centrifuged at 4000 rpm for 10 min, and the supernatant was discarded. Insoluble residue was dissolved with 50 ml of methanol, and the solution was filtered through a sintered glass filter. The volume was reduced by rotary evaporation, and the concentrated solution was applied to a preparative HPLC with a YMC-Pack ODS column (20×250

*To whom correspondence should be addressed.
E-mail: adachi@kais.kyoto-u.ac.jp

mm) to purify the product. Analysis of the product followed by ^{1}H NMR: (500 MHz, CD$_3$OD, 297 K, TMS) (ppm): 0.89 (3H), 1.29 (16H), 1.61 (2H), 2.37 (2H), 3.24 (1.5H), 3.46 (3H), 3.60 (1.5H), 3.86 (4H), 4.16 (1H), 4.37 (1H), 4.50 (0.7H), 5.12 (1.5H). Although this analysis indicated that the product was a monolauroyl maltose, it could not be concluded whether the 6-*O*- or 6-*O′*-hydroxyl group of maltose was acylated. Woudenberg-van Oosterom *et al.* (1996) reported that *Candida antarctica* lipase catalyzed the acylation of only the 6-*O′*-hydroxyl group of maltose. As shown later, the residual area per molecule of the product, which was determined from the measurement of the surface tensions of the aqueous solution of the product, suggested acylation of maltose at the 6-*O′*-hydroxyl group of maltose.

Continuous synthesis of monolauroyl maltose using a CSTR
A glass vessel with a stainless steel lid was used as the CSTR. The lid had tubes for supplying a solution of lauric acid, withdrawing the reaction mixture and circulating the mixture or air in the reactor. Immobilized-lipase particles (24 g) were packed into a stainless steel basket which had an opening of 0.2 mm and was fixed to the lid. At the beginning of the operation, 15 mmol of maltose monohydrate, 60 mmol of lauric acid and 300 ml of acetone were added to the reactor, and the lid was tightly clamped down. Lauric acid dissolved in acetone at a concentration of 200 mmol/*l* was fed to the reactor using an MP-3N peristaltic pump (Tokyo Rikakikai, Tokyo) at a flow rate of 0.2 ml/min, and the reaction mixture was withdrawn at the same flow rate, which corresponded to a residence time of 25 h. The reactor was immersed in a water bath at 50°C and the reaction mixture was mixed by a magnetic stirrer. The product concentration in the effluent was determined by the method described above.

The maltose used as a substrate was a monohydrate. The removal of water brought in with the maltose and produced through its condensation would promote the formation of lauroyl maltose. Therefore, water was removed by the following three procedures: 1) gas in the headspace of the reactor was circulated through a glass column packed with 24 g of 4A molecular sieves at a flow rate of 6 ml/min, 2) the reaction mixture was circulated through the column at a flow rate of 4 ml/min, and 3) 24 g of molecular sieves, which were packed into a stainless steel basket, was directly immersed into the reaction mixture. In every case, the molecular sieves were replaced with new ones every 24 h.

Solubility of maltose in acetone Maltose monohydrate (90.1 mg) was weighed in a vial, and 5 ml of dehydrated acetone was added. The vial was tightly screw-capped and immersed in a water bath at 50°C. After 24 h, the solution phase was carefully sampled to assure it was not contaminated by undissolved maltose. The maltose concentration in the sample was analyzed by HPLC with a YMC-pack NH$_2$ column (4.6×250 mm, Kyoto) and a RID-6A refractometer. The eluent was a mixture of acetonitrile and water (70 : 30 by vol.) and had a flow rate of 1.0 ml/min.

Surface tension of aqueous solutions of monolauroyl mannose
Monolauroyl maltose was dissolved in distilled water at concentrations of $2.5×10^{-7}$ to $1.0×10^{-3}$ mol/*l*. The surface tension of the solution was measured by the Wilhelmy method using a CBVP-A3 surface tensiometer (Kyowa Kaimenkagaku, Tokyo) at 25, 32 and 39°C.

Results and Discussion

Maltose and lauric acid were condensed in a batch reactor at 50°C and their initial concentrations of 50 mmol/*l* and 100 mmol/*l*, respectively, in four water-miscible organic solvents, acetone (0.030), acetonitrile (0.018), 2-methyl-2-propanol (0.013) and 2-methyl-2-butanol (0.009), in the absence of the 4A molecular sieves. The figures in parentheses were the conversion at the reaction time of 24 h. Under these conditions, the conversions of maltose to monolauroyl maltose at 48 h were low (about 0.01 to 0.02). Because the highest conversion was obtained in acetone among all the solvents tested and acetone is approved for use in food processing in many countries or areas, acetone was selected as the reaction medium.

Figure 1 (a) and (b) show the effect of the molar ratio of lauric acid to maltose on the conversion in the absence and presence (60 mg/ml) of 4A molecular sieves, respectively. The initial concentration of maltose was fixed at 50 mmol/*l*. When no molecular sieves were added to the reaction mixture, the conversion was low at all molar ratios. Addition of the 4A molecular sieves to the mixture significantly increased the conversion. The conversion of *ca.* 0.4 at 72 h was achieved at the molar ratios of 4 and 6.

The effect of the amount of 4A molecular sieves on the conversion was examined at the initial concentrations of maltose and lauric acid of 50 mmol/*l* and 200 mmol/*l*, respectively (Fig. 2). The conversion at 96 h or later was higher as more 4A molecular sieves were added to the reaction mixture. This indicated the removal of water by the desiccant was effective in raising the conversion during the condensation. However, the conversion slightly decreased at the 100 mg-molecular sieves/ml; one possi-
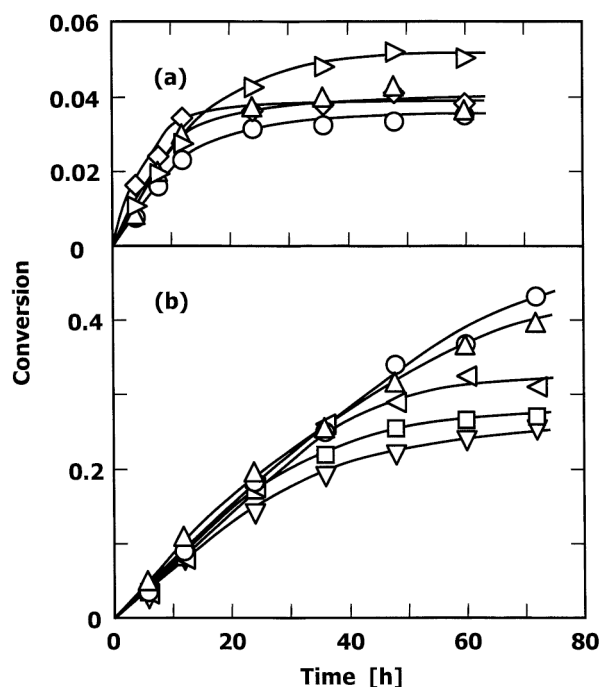
Fig. 1. Condensation of maltose and lauric acid in a batch reactor at various molar ratios of lauric acid to maltose in the (a) absence and (b) presence of 4A molecular sieves. The initial maltose concentration was fixed at 50 mmol/*l*. Acetone was used as the reaction medium, and the amount of immobilized lipase used was 100 mg in 5 ml of acetone. Condensation was carried out at 50°C. The molar ratios were (▽) 1, (□) 2, (◁) 3, (○) 4, (◇) 5, (△) 6 and (▷) 8.

ble reason for the decrease might have been the formation of diester.

The product concentration is important in practice as is the conversion. The condensation was carried out at various maltose concentrations. The molar ratio of lauric acid to maltose was fixed at 4. Because the amount of water brought in with the maltose monohydrate was proportional to that concentration, the amount of 4A molecular sieves was increased proportionally to the maltose concentration. That is, the ratio of the amount of molecular sieves to maltose was fixed at 1600 mg-molecular sieves/mmol-maltose. As shown in Fig. 3, the condensation proceeded faster at lower maltose concentration. At an initial maltose concentration of 50 mmol/*l*, the product concentration reached a plateau (30 mmol/*l*) corresponding to a conversion of

0.6. When the maltose concentration was 100 mmol/*l*, the product concentration increased almost linearly with reaction time and surpassed that at the maltose concentration of 50 mmol/*l* on day 5. At higher maltose concentrations, the reaction rate was very low. In these experiments, most of the maltose was not dissolved but was dispersed in the acetone. Only dissolved maltose can be useful as substrate for the lipase. The solubility of maltose in acetone at 50°C was 0.41 mmol/*l*, which was much lower than the overall maltose concentration. The concentration of maltose dissolved in acetone seemed to remain constant. However, because the molar ratio of lauric acid to maltose was fixed at 4, there was a possibility that the concentration of dissolved maltose was lower at the higher overall concentration due to the greater amount of lauric acid and that the reaction rate decreased
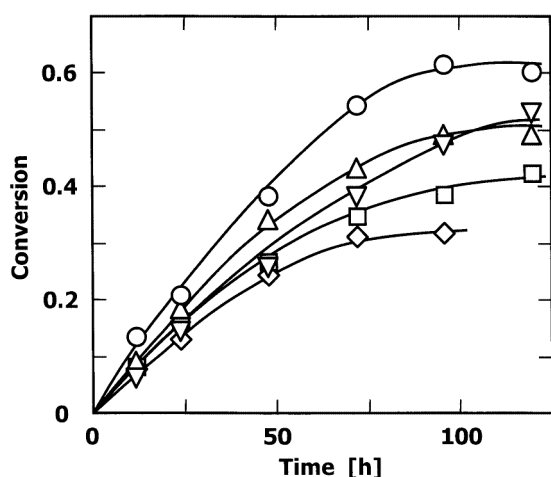

Fig. 2. Effect of the amount of 4A molecular sieves on the conversion in a batch reactor. The initial concentrations of maltose and lauric acid were 50 and 200 mmol/*l*, respectively. The amount of immobilized lipase and the temperature were the same as in Fig. 1. The amounts of molecular sieves per ml-acetone were (◇) 20, (□) 40, (△) 60, (○) 80 and (▽) 100 mg.

Fig. 4. Synthesis of monolauroyl maltose at 50°C using a continuous stirred reactor. Three different procedures were used to remove water from the reaction medium: (○) a stainless steel basket packed with 4A molecular sieves was directly immersed in the reaction mixture, (△) the reaction mixture was circulated through a glass column packed with 4A molecular sieves, and (□) gas in the headspace in the reactor was circulated through the column.
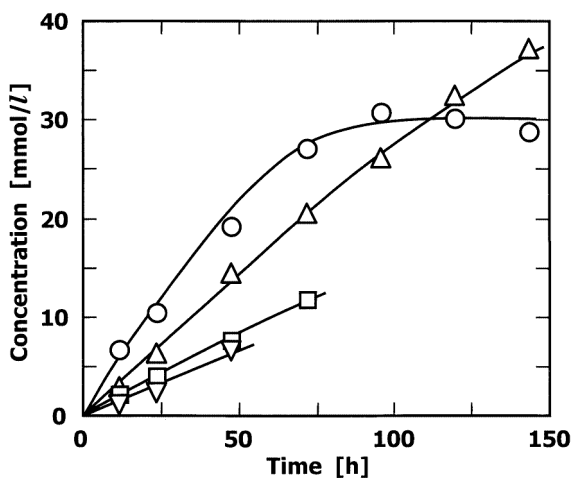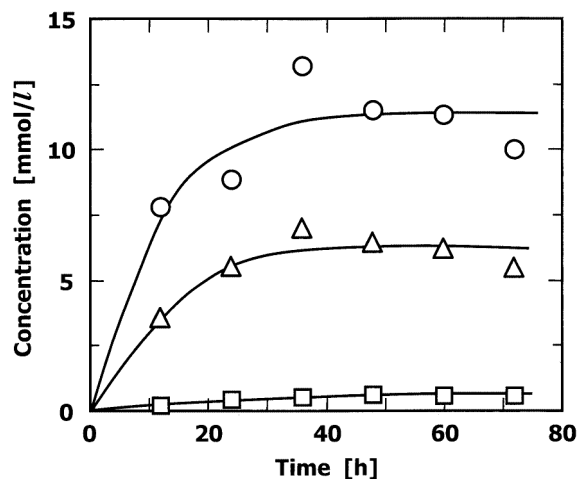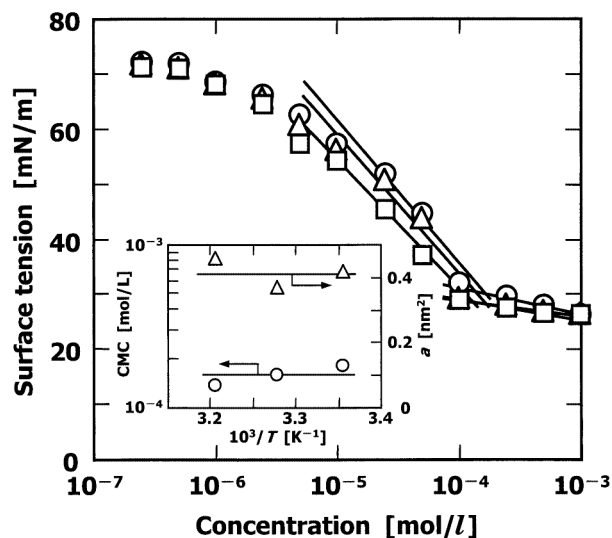

Fig. 3. Changes in the product concentration with reaction time at various initial maltose concentrations in a batch reactor. The molar ratio of lauric acid to maltose was fixed at 4, and the amount of 4A molecular sieves was changed to produce a fixed ratio of the molecular sieves to maltose (1600 mg/mmol-maltose). The amount of immobilized lipase and the temperature were the same as in Fig. 1. The initial maltose concentrations were (○) 50, (△) 100, (□) 150 and (▽) 200 mmol/*l*.

Fig. 5. Surface tensions of aqueous solution of monolauroyl maltose at various concentrations and at (○) 25, (△) 32 and (□) 39°C. Inset shows the plots of the critical micelle concentration, CMC, and the residual area per molecule, *a*, versus the reciprocal of the absolute temperature, 1/*T*.

at the higher maltose concentration.

Monolauroyl maltose was continuously synthesized using a CSTR (Fig. 4). Among the three procedures attempted to remove water, the direct immersion of 4A molecular sieves into the reaction mixture was the most effective and a product concentration of *ca*. 12 mmol/*l* was achieved after an operation time of 30 h. The circulation of gas in the headspace of the reactor was ineffective for removing water.

Figure 5 shows the relationships of the surface tension of the aqueous solution of monolauroyl maltose and its concentration at various temperatures. The critical micelle concentration, CMC, was estimated from the intersection of the two lines at each temperature. The surface excess was evaluated from the slope of the line drawn at the low concentrations, and the residual area per molecule, *a*, was calculated from the surface excess. As shown in the inset, the CMC value depended little on the temperature. The *a* value was *ca*. 0.4 nm^2. The molar volumes of maltose and glucose at 25°C were 0.219 and 0.114 *l*/mol, respectively (Adachi & Matsuno, 1997). If these molecules are assumed to be spherical, their cross-sectional areas are 0.61 and 0.40 nm^2, respectively. Therefore, we can hypothesize that the maltose residue of monolauroyl maltose is almost vertically situated in the aqueous solution at the air-aqueous interface.

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