Note

Surfactant Properties of Enzymatically Synthesized Monolauroyl Disaccharides

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Monolauroyl maltose, palatinose, trehalose and sucrose were synthesized by *Candida antarctica* lipase-catalyzed condensation in an organic solvent, and their surfactant properties were measured at 25°C. The type of hydrophilic moiety of the lauroyl disaccharides did not significantly affect the critical micelle concentration, the surface tension at this concentration, residual area per molecule, free energy of micellization or micelle size, but produced different solubilization ability against octanoic acid.

Keywords: surface activity, nonionic surfactant, lipase, monolauroyl disaccharide

Introduction

The sucrose fatty acid ester is a nonionic surfactant and has been widely used in the food, cosmetic and pharmaceutical fields due to its biodegradability and biocompatibility (Baker *et al.*, 2000). Fatty acid esters of other di-, mono- and trisaccharides have also been shown to be surface active (Söderberg *et al.*, 1995; Garofalakis *et al.*, 2000; Kjellin *et al.*, 2001; Douglas and Tao, 2002; Ferrer *et al.*, 2002; Zhang *et al.*, 2003; Chen *et al.*, 2005; Chen *et al.*, 2007; Piao *et al.*, 2007; Sun *et al.*, 2009). Recently, enzymatic synthesis in organic solvents has attracted attention as an alternative to conventional chemical synthesis of esters (Carrea and Riva, 2000). In particular, lipase from *Candida antarctica* preferentially acylates primary hydroxyl groups, and the lipase is used in the condensation of mono-, di- and trisaccharides with various fatty acids (Cao *et al.*, 1999; Degn *et al.*, 1999; Zhang *et al.*, 2003; Soultani *et al.*, 2003; Chen *et al.*, 2005; Chen *et al.*, 2007; Piao *et al.*, 2007; Sun *et al.*, 2009).

It has been reported that the surface activity of the sugar fatty acid esters is affected by the type of sugar head group, the length of the acyl chain and the composition of the esters (Garofalakis *et al.*, 2000; Ferrer *et al.*, 2002; Soultani *et al.*, 2003; Chen *et al.*, 2007; Imura *et al.*, 2010; Schiefelbein *et al.*, 2010). The effect of the sugar head group on the surfactant properties has usually been investigated for the esters of saccharides having different molecular masses, such as the mono-, di- and trisaccharides. The difference in the molecu-

lar mass of the hydrophilic moiety of the esters produces a difference in their hydrophilic-lipophilic balance (HLB) and hence affects their surfactant properties. Furthermore, most of the reports have dealt with only the surface tension and the critical micelle concentration (CMC), while the micelle size or solubilizing capacity has been scarcely investigated. Solubilized solutions are colorless, transparent and thermodynamically stable. Solubilization also enables the suppression of lipid oxidation and flavor deterioration, and improves flavor by lipophilic aromatic compounds. Therefore, solubilization is a useful technique in food. However, the amount of solubilized substance is limited because solubilized particle sizes are usually smaller than 10 nm. In this context, evaluation of the solubilizing capacity of surfactants and of the factors affecting solubilization are important.

In this study, we synthesized the lauroyl esters of several disaccharides using *Candida antarctica* lipase in an organic solvent, and examined whether the surfactant properties of these esters, which have the same HLB number, were affected by the disaccharide type. The disaccharides used were maltose, palatinose, trehalose and sucrose. The surface tensions were measured at 25°C to estimate the CMC and the surface tensions at the CMC, which are general surfactant properties. The micelle size and solubilizing capacity of these esters against octanoic acid were also measured.

Materials and Methods

Materials Immobilized lipase from *Candida antarctica* (Chirazyme® L-2 c.-f. C-2) was purchased from Roche Molecular Biochemicals (Mannheim, Germany). Maltose

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monohydrate and trehalose dihydrate were purchased from Hayashibara, Okayama, Japan. Lauric acid, palatinose monohydrate, and sucrose were purchased from Wako Pure Chemical Industries, Osaka, Japan. All other chemicals were purchased from Wako Pure Chemical Industries.

Enzymatic synthesis and purification of monolauroyl disaccharides The monolauroyl esters of maltose, palatinose, trehalose and sucrose were synthesized according to our previous procedures (Piao *et al.*, 2007) with some modifications. The disaccharide (50 mmol) and lauric acid (100 mmol) were weighed into a glass bottle, then dissolved or suspended in a mixture of *tert*-butyl alcohol and pyridine (8/2 by vol., 500 mL). The immobilized lipase (5 g) was put into a nylon-mesh bag and added to the bottle. The bottle was tightly screw-capped and immersed in a water bath at 60°C with shaking at *ca.* 120 strokes/min to start the condensation reaction. After 48 h, the reaction mixture was sampled and the formation of the lauroyl disaccharides using HPLC was evaluated. The HPLC was equipped with an LC-10AD pump (Shimadzu, Kyoto, Japan), a 5C18-AR-II column (4.6 mm ID×150 mm, Nacalai Tesque, Kyoto) and an SPD-10AV UV detector (Shimadzu). The eluent was a mixture of isopropyl alcohol, acetonitrile and water (33/30/37 by vol.) at a flow rate of 0.8 mL/min.

The reaction mixture was filtered through No. 2 filter paper (Toyo Roshi, Tokyo, Japan) to remove any undissolved disaccharide. The filtrate was rotary-evaporated under reduced pressure to remove the solvent. Hexane was added to the remainder to dissolve lauric acid and the hexane phase was removed by decantation. Acetone was then added to the remainder to dissolve the esters and the acetone phase was obtained by filtration. The acetone solution was then rotary-evaporated under reduced pressure to recover the esters. The condensation products were confirmed to be the desired ones based on their ¹H NMR spectra. The ¹H NMR spectra of the products were as follows. Monolauroyltrehalose: δ (ppm, 500 MHz, CD₃OD) 0.90 (3H, t, $J = 7.1$ Hz), 1.29 (16H, m), 1.61 (2H, m), 2.34 (2H, t, $J = 7.4$ Hz), 3.35 (2H, m), 3.47 (2H, m), 3.67 (1H, m), 3.74–3.86 (4H, m), 4.01 (1H, m), 4.20 (1H, m), 4.36 (1H, m), 5.08 (2H, m). Monolauroylsucrose: δ (ppm, 500 MHz, CD₃OD) 0.90 (3H, t, $J = 7.1$ Hz), 1.29 (16H, m), 1.61 (2H, m), 2.35 (2H, m), 3.25 (1H, m), 3.40 (1H, m), 3.56–3.84 (5H, m), 3.91–4.20 (4H, m), 4.30–4.55 (2H, m), 5.38 (1H, m). Monolauroylmaltose: δ (ppm, 500 MHz, CD₃OD) 0.90 (3H, t, $J = 7.1$ Hz), 1.29 (16H, m), 1.61 (2H, m), 2.37 (2H, t, $J = 7.4$ Hz), 3.26 (1H, m), 3.38–3.50 (3H, m), 3.63 (1H, m), 3.75–3.92 (5H, m), 4.17 (1H, m), 4.39 (1H, m), 5.10 (2H, m). Monolauroylpalatinose: δ (ppm, 500 MHz, CD₃OD) 0.90 (3H, t, $J = 7.1$ Hz), 1.29 (16H, m), 1.60 (2H, m), 2.34 (2H, t, $J = 7.5$ Hz), 3.31 (1H, m), 3.40 (1H, m), 3.47 (1H,

m), 3.55–3.72 (3H, m), 3.78–3.92 (3H, m), 3.96 (1H, m), 4.06 (1H, m), 4.14 (1H, m), 4.21 (1H, m), 4.37 (1H, m).

Surface tension Each monolauroyl disaccharide was dissolved in distilled water at various concentrations. The surface tensions of the solutions were measured by the drop weight method at 25°C. The measurement was conducted in triplicate under specific conditions, and the values were averaged.

Micelle size The size distribution of the micelles in a monolauroyl disaccharide solution was measured at 25°C using a DLS-7000 dynamic light scattering spectrophotometer (Otsuka Electronics, Osaka, Japan) to estimate the median diameter based on the weight fraction. The diameter was measured at various concentrations of each monolauroyl disaccharide.

Solubilizing capacity Octanoic acid was chosen as the solubilized compound. A monolauroyl disaccharide solution dissolved in distilled water at various concentrations (5 mL each) was added to the vials. The appropriate amount of octanoic acid was then added to the vials. The vials were immersed in a water bath at 25°C with shaking at *ca.* 160 strokes/min. After achieving equilibrium, the absorbance of the solutions was measured using a UV-1200 UV-VIS spectrophotometer (Shimadzu) at 500 nm.

Results and Discussion

Surface activities of monolauroyl disaccharides Figure 1 shows the surface tension curves of the monolauroyl disaccharides at 25°C. The CMC was estimated from the intersection of the two lines for each ester. The surface tension at the CMC, γ_{CMC} , was also determined from the intersection. The surface excess, Γ , was evaluated from the slope of the line drawn at the low concentrations according to the Gibbs equation:

$$\frac{d\gamma}{d \log C} = -2.30\Gamma RT \quad (1)$$

where γ is the surface tension, C is the concentration, R is the gas constant and T is the absolute temperature. The residual area per molecule, a , was calculated from Eq. (2).

$$a = 1/(N_A \Gamma) \quad (2)$$

where N_A is Avogadro's number. The free energy of micellization, ΔG_m , is given by Eq. (3).

$$\Delta G_m = RT \ln \text{CMC} \quad (3)$$

The surfactant properties of the monolauroyl maltose, palatinose, trehalose and sucrose at 25°C are summarized in Table 1. It was reported that the CMC value of the monolauroyl trehalose was 0.56 mmol/L at 30°C (Chen *et al.*, 2007), those of the monolauroyl maltose were 0.24 mmol/L at 25°C

Fig. 1. Surface tension curves of monolauroyl maltose (○), palatinose (◇), trehalose (△) and sucrose (□) at 25°C.

Fig. 2. Concentration dependence of the micelle size of monolauroyl maltose, palatinose, trehalose and sucrose at 25°C. The symbols are the same as in Fig. 1.

Table 1. Surface activities of monolauroyl disaccharides at 25°C.

Saccharide	CMC [mmol/L]	γ_{CMC} [mN/m]	$\Gamma \times 10^6$ [mol/m ²]	a [nm ²]	ΔG_m [kJ/mol]
Maltose	0.27	36.3	7.15	0.23	-20.3
Palatinose	0.42	33.5	7.30	0.23	-19.3
Trehalose	0.54	42.3	6.11	0.27	-18.7
Sucrose	0.31	43.0	6.20	0.27	-20.0

(Ferrer *et al.*, 2002) and 0.33 mmol/L at 22°C (Kjellin *et al.*, 2001), and those of the monolauroyl sucrose were 0.45 mmol/L at 20°C (Söderberg *et al.*, 1995), 0.25 mmol/L at 25°C (Ferrer *et al.*, 2002), and 0.21 mmol/L at 32°C (Garofalakis *et al.*, 2000). The CMC values of the monolauroyl disaccharides synthesized in this study were almost the same as the reported ones. There were no significant differences in the CMC, Γ , a and ΔG_m among the four monolauroyl disaccharides tested here, and a similar tendency has also been reported for the monomyristoyl disaccharides (Piao *et al.*, 2007). As shown in Table 1, the γ_{CMC} value depended slightly on the type of disaccharide. These results indicated that the type of hydrophilic moiety of the acyl disaccharides does not significantly affect their surfactant properties.

Micelle size of monolauroyl disaccharides The micelle sizes of the monolauroyl disaccharides were measured at various concentrations and at 25°C (Fig. 2). Because each monolauroyl disaccharide had a slightly different CMC value, the concentrations were normalized by the CMC value in the figure. When the concentration was beyond the CMC, the micelle size increased with increasing concentration. The micelle size of the monolauroyl maltose increased more

gradually compared with those of other monolauroyl disaccharides. This was ascribed to the lower CMC value of the monolauroyl maltose. For all the monolauroyl disaccharides, the micelle size became *ca.* 7 nm at concentrations 2 to 3 times higher than their CMC values, i.e., 0.7 to 1.0 mmol/L, and no further increase in the micelle size was observed at higher concentrations. Thus, the micelle size was also not affected by the type of hydrophilic moiety of the lauroyl disaccharides.

Solubilization ability of monolauroyl disaccharides The solubilizing capacity of each monolauroyl disaccharide was assessed by measuring the amount of octanoic acid solubilized into the micelles at 25°C. Figure 3 shows the absorbance at 500 nm of the monolauroyl palatinose solutions to which various amounts of octanoic acid had been added. The absorbance abruptly increased when the amount was beyond a certain level. The maximum solubilizing capacity was estimated from the intersection of the two lines drawn at the low and high concentrations.

Figure 4 shows the concentration of octanoic acid solubilized into the micelle at the various surfactant (monolauroyl disaccharide) concentrations for the four monolauroyl di-

Fig. 3. Examples of octanoic acid solubilization of monolauroyl palatinose at 25°C. The monolauroyl palatinose concentration was 0.1 ($\diamond$), 0.2 ($\circ$), 0.3 ($\triangle$) and 0.4 mmol/L ($\square$).

saccharides. The slope of the line denotes the solubilization ability of the surfactant, with a higher slope indicating higher ability. The slopes of the monolauroyl maltose, palatinose, trehalose and sucrose were 0.88, 1.2, 2.0 and 2.0, respectively. The monolauroyl trehalose and sucrose had the highest ability, followed by monolauroyl palatinose and maltose. It is generally recognized that the factors affecting the ability are the acyl chain length, micelle size, ionicity of the surfactant, and temperature (Nagarajan, 1996). The esters tested in this study were all nonionic, with the same acyl chain and almost the same micelle size. The measurements were also conducted at the same temperature. Therefore, it is suggested that the hydrophilic moiety type affects the solubilization ability even if the molecular mass of the moiety is the same. The reason for the different ability is unexplained, but it may be ascribed to the weak surface activity of octanoic acid.

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Fig. 4. Octanoic acid solubilization ability of monolauroyl maltose, palatinose, trehalose and sucrose at 25°C. The symbols are the same as in Fig. 1.

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