Glucoside Ester Synthesis in Microemulsions Catalyzed by *Candida antarctica* Component B Lipase

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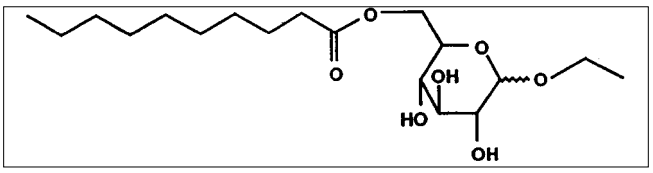
ABSTRACT: The surfactant, ethyl 6-*O*-decanoyl glucoside, was synthesized in microemulsion systems by lipase catalysis. The microemulsions were based on the two substrates for the reaction, ethyl glucoside and fatty acid, and either the sodium salt of the fatty acid or the glucoside ester was used as surfactant. The lipase used was component B from *Candida antarctica.* Reduced pressure was employed to eliminate the water of condensation. The reaction yield was good, with conversion of fatty acid and ethyl glucoside reaching 77 and 96%, respectively.
JAOCS 74, 39–42 (1997).

KEY WORDS: *Candida antarctica,* condensation, esterification, ethyl 6-*O*-decanoyl glucoside, fatty acid, glucoside ester, lipase, microemulsion, self-diffusion NMR.

Surfactants of good biodegradability and low toxicity have been the subject of considerable interest in recent years (1–10). Sugar esters prepared by condensation of a fatty acid with a mono- or disaccharide are examples of nonionic surfactants as seen in the structure of the glucoside ester, ethyl 6-*O*-decanoyl glucoside (Scheme 1). Lipase-catalyzed condensation of a sugar with a fatty acid in aqueous medium has previously been described, but the reported yields have been low (5). Higher yields can be obtained in various organic solvent-based reaction systems, such as pyridine (6) or tertiary butyl alcohol (7), or by using a two-phase system that consists of sugar dissolved in water and fatty acid (8) or ethyl glucoside dissolved in melted fatty acid, with immobilized lipases (9). To obtain high yields, the choice of reaction system is important. If enzymes are used as catalyst, the system must provide suitable conditions for the biocatalyst. The process must be designed to give easy recovery of the product, and it should also be environmentally safe. The aim of the present work was to improve the process by using a microemulsion as reaction medium.



SCHEME 1

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It has been shown earlier that microemulsions are suitable media for lipase-catalyzed reactions, particularly when one substrate is oil-soluble and the other substrate is water-soluble. The reaction yield depends on the type of surfactant used in the microemulsion formulation (11). Most lipase-catalyzed esterification reactions in microemulsions have been performed in the presence of Aerosol-OT [sodium *bis*(2-ethylhexyl)sulfosuccinate] or some other amphiphile that does not take an active part in the enzymatic process. It must be separated from the product after completion of the reaction. One exception is the work of Singh *et al.* (12) in which the microemulsion surfactant was the substrate for the reaction. The surfactants used in the present work consisted of either the substrate, i.e., fatty acid/fatty acid soap, or the product, a glucoside ester. Hence, the amphiphiles do take an active part in the enzymatic reactions. The only other components of the system were ethyl glucoside and water. The reaction was catalyzed by *C. antarctica* component B lipase.

Water activity is an important parameter for condensation reactions. If the water activity is too high, hydrolysis will be favored. Hence, it is necessary to control water activity, for example by applying vacuum during the reaction (9) or by using a high sugar-to-water ratio in the water phase. In the present study, we have used vacuum during the course of the reaction.

MATERIALS AND METHODS

Materials. Partially purified *C. antarctica* component B lipase [250 lipase unit (LU)/mg], ethyl glucoside, and ethyl 6-*O*-decanoyl glucoside were prepared by Novo Nordisk A/S (Bagsvaerd, Denmark). One LU corresponds to the amount of enzyme that liberates one micromole butyric acid per minute from a tributyrin substrate at a temperature of 30°C and a pH of 7.0. Decanoic acid and chloroform were purchased from Merck (Darmstadt, Germany). Sodium decanoate was purchased from Fluka (Buchs, Switzerland).

Phase diagrams. Technical partial phase diagrams were determined for two systems, based on sodium decanoate/decanoic acid/water and on ethyl 6-*O*-decanoyl glucoside/decanoic acid/water at different ethyl glucoside concentrations. The temperature was 50°C.

Microemulsions and reaction conditions. Decanoic acid and ethyl glucoside were co-solubilized by stirring and heating to 50°C. Sodium decanoate was added, and the sample was stirred in a flask (microemulsions 4 and 5) or rotated in a rotavapor (Büchi, Flamil, Switzerland) at 220 rpm (microemulsions 6 and 7) at 50°C until the solutions became clear. The reaction was started by addition of a solution that consisted of 20 mg lipase/mL water. The total amount of a sample was typically 5 g. The compositions of the microemulsions are listed in Table 1. The synthesis reaction taking place in the microemulsions is:

$$\text{ethyl glucoside + decanoic acid} \overset{\text{lipase}}{\rightleftarrows} \text{ethyl 6-}O\text{-decanoyl glucoside + water} \quad [1]$$

The reactions were run at 50 ± 0.5°C at atmospheric pressure (microemulsion 4) or under reduced pressure, 0.2 bar (microemulsions 5–7).

Yield measurements. After completion of incubation, ethanol was added (ethanol/reaction mixture, 99:1, w/w). The consumption of ethyl glucoside in microemulsions 4 and 5 was determined by high-performance liquid chromatography (HPLC) with an amino-propyl column (Hewlett-Packard, Kista, Sweden) and 99.5% ethanol as mobile phase. A refractive index detection unit (Hewlett-Packard) was used. The consumption of decanoic acid in microemulsions 6 and 7 was determined by fatty acid titration with 0.1 M KOH in ethanol.

*Nuclear magnetic resonance (NMR) identification of ethyl 6-*O*-decanoyl glucoside product.* The structure of ethyl 6-*O*-decanoyl glucoside was confirmed by NMR with a 400 MHz Bruker apparatus (Bruker Spectrospin AB, Täby, Sweden). The ^{1}H NMR spectroscopic data for a sample of the reaction mixture after 24 h reaction time is given below. In comparison with the NMR spectra from decanoic acid and ethyl glucoside, there are new peaks at 4.2 and 4.3 ppm, which are compatible with an ester structure at the 6-position. The ^{1}H NMR spectroscopic data are in accordance with previously published data (9). ^{1}H NMR ($CDCl_3$), ppm: 0.9, 1.2, 1.6, 2.2, 3.3, 3.5, 3.7, 3.9, 4.1, 4.2, 4.3, 4.8.

Self-diffusion measurements. Self-diffusion coefficients were obtained by the ^{1}H NMR Fourier transform pulsed-gradient spin-echo (FT-PGSE) technique in a standard Jeol (Tokyo, Japan) FX-100 NMR spectrometer, operating at a proton frequency of 99.6 MHz and at a temperature of 50 ± 1°C. The PGSE measurements were performed by varying the duration of the gradient pulse at a constant measuring time of 140 ms (13).

Product separation. After completion of incubation, ethanol was added, pH was adjusted to 3 with HCl, and 100 mL chloroform was added. The chloroform solution was washed four times with 200 mL of a 2% (w/w) bicarbonate solution. The mixture was separated, and the chloroform solution was evaporated. The remaining amount of fatty acid was determined by titration.

RESULTS AND DISCUSSION

Phase diagrams with the fatty acid substrate as surfactant and self-diffusion NMR measurements. Figure 1 shows a technical partial phase diagram for water, fatty acid, and fatty acid solium salt at different contents of ethyl glucoside and glucoside ester. The phase diagram was determined to investigate if there were any major changes in the extent of the oil-continuous microemulsion phase, the L2-phase, as a consequence of an increasing concentration of the surface-active glucoside ester during the course of the reaction. As can be seen, the L2-phase was relatively large in all systems. A minimum amount of sodium decanoate, 5–10% (w/w), was needed to form a microemulsion that contained equal parts of water and fatty acid. As shown in Figure 1, the microemulsion phase extended further toward the corner of the fatty acid soap with increasing amounts of the glucoside ester.

The L2-phase of the system that consisted of water, sodium decanoate, and decanoic acid was characterized by self-diffusion NMR measurements of water. The self-diffusion data and compositions of the microemulsions studied are shown in Table 2. The self-diffusion of water increased with increasing concentration of water. Only the sample with the

TABLE 1
Compositions (w/w) for the Microemulsions Used, Conversion of Decanoic Acid and Ethyl Glucoside, and Calculated Conversion of Decanoic Acid and Ethyl Glucoside After 24 h Incubation

			Microemulsion composition (%)[a]						
System	Pressure (atm)	LU/g microemulsion	Water	Sodium decanoate	Ethyl 6-*O*-decanoyl glucosde	Decanoic acid	Ethyl glucoside (%)[b]	Conversion of decanoic acid (%)	Conversion of ethyl glucoside (%)
Microemulsion 4	1.0	850	17	17		66	40	25[c]	30
Microemulsion 5	0.2	850	17	17		66	40	67[c]	80
Microemulsion 6	0.2	350	7		6	87	46	35	44[d]
Microemulsion 7	0.2	1400	28		12	60	37	77	96[d]

[a]Calculated on the components water, sodium decanoate or ethyl 6-*O*-decanoyl glucoside and decanoic acid; LU, lipase unit. [b]Calculated on the total amount of sample. [c]Calculated from the conversion of ethyl glucoside. [d]Calculated from the conversion of decanoic acid.

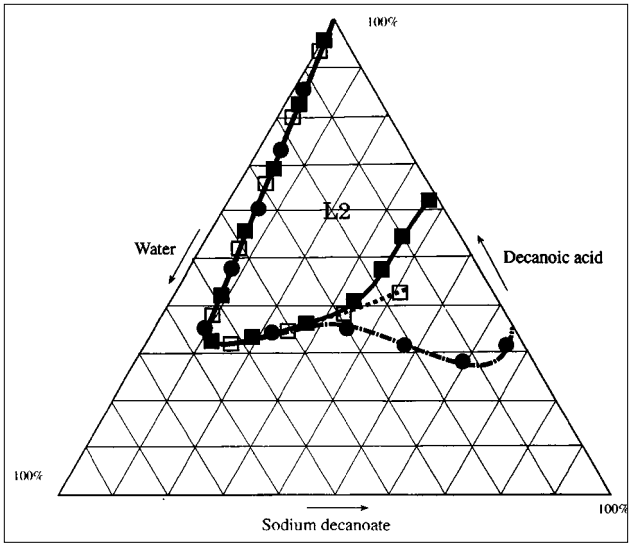

FIG. 1. Technical partial phase diagrams (w/w) of water, sodium decanoate, and decanoic acid in the presence of (■) 40% ethyl glucoside, (□) 24% ethyl glucoside and 35% ethyl 6-*O*-decanoyl glucoside, (●) 8% ethyl glucoside and 70% ethyl 6-*O*-decanoyl glucoside. L2 denotes an oil-continuous microemulsion. The temperature was 50°C.

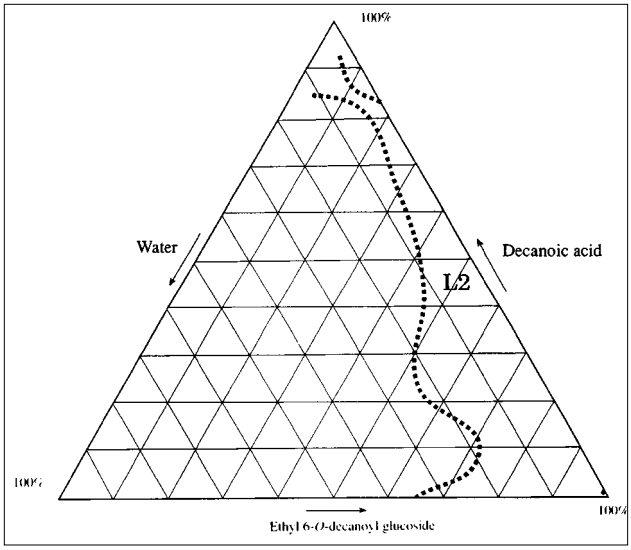

FIG. 2. Technical partial phase diagram for water, ethyl 6-*O*-decanoyl glucoside and decanoic acid. L2 denotes an oil-continuous microemulsion. The temperature was 50°C.

lowest water concentration showed a typical oil-in-water microemulsion structure. Self-diffusion coefficients for water for three different microemulsions/self-diffusion coefficient for water in a water solution with 33% (w/w) ethyl glucoside ($D_w/D°_w$) values of the other two samples are indicative of a more bicontinuous microemulsion structure.

Phase diagram with the product as surfactant. Figure 2 shows the technical partial phase diagram of water, fatty acid, and the glucoside ester. It is clearly seen that the microemulsion region extends to the surfactant corner. Accordingly, it is possible to make the reaction proceed to 100% conversion of the fatty acid within the microemulsion phase.

Condensation reactions. Condensation of fatty acid with ethyl glucoside, catalyzed by *C. antarctica* component B lipase, was carried out in microemulsions of different compositions (Table 1). All compositions were within the isotropic L2-phase except for microemulsion 7, which was a two-phase system that consisted of one water phase and one L2-phase in equilibrium. Microemulsions 4 and 5 could be indicated in the phase diagram, Figure 1, because they contain 40% ethyl glucoside while microemulsions 6 and 7 do not. However, from different trials, the concentration of ethyl glucoside seemed not to be as crucial as the concentration of water for the phase behavior. Therefore, the phase behavior could be estimated from Figure 1 independent of the ethyl glucoside concentration.

Figure 3 shows the conversion of ethyl glucoside as a function of reaction time in the microemulsions with fatty acid sodium salt as surfactant. The conversion of ethyl glucoside after 24 h reaction time for microemulsions 4–7 is also shown

TABLE 2
Compositions (w/w) and Self-Diffusion Coefficients for Water, D_w, for Three Different Microemulsions

System	Microemulsion composition (%)[b]			Ethyl glucoside (%)[c]	$D_w/D_w°$
	Water	Sodium decanoate	Decanoic acid		
Microemulsion 1	14	30	56	36	0.03
Microemulsion 2	25	25	50	33	0.1
Microemulsion 3	33	22	45	31	0.2

[a]$D°_w$ is the self-diffusion coefficient for water in a water solution with 33% (w/w) ethyl glucoside.
[b]Calculated on the three components water, sodium decanoate, and decanoic acid.
[c]Calculated on the total amount of sample.

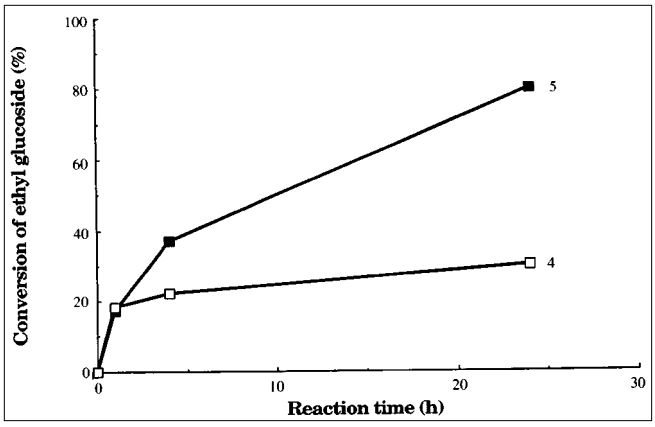

FIG. 3. Conversion of ethyl glucoside in microemulsions, based on the fatty acid sodium salt as surfactant, vs. reaction time. The microemulsion composition is shown in Table 2. The temperature was 50°C and atmospheric pressure (4) or reduced pressure, 0.2 bar (5), was used. The concentration of *Candida antarctica* component B lipase was 850 lipase unit/g microemulsion.

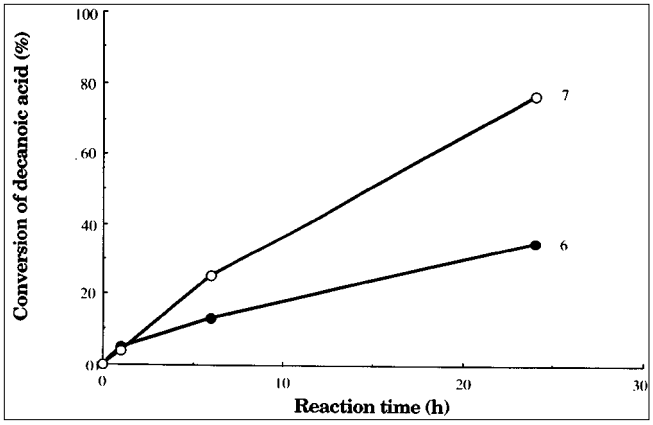

FIG. 4. Conversion of decanoic acid in microemulsions, based on the product as surfactant, vs. reaction time. The microemulsion composition is given in Table 2. The temperature was 50°C and reduced pressure, 0.2 bar, was used. The concentration of *Candida antarctica* component B lipase was 350 lipase unit/g (6) or 1400 lipoase unit/g microemulsion (7).

in Table 1. Microemulsion 4 was run at atmospheric pressure, whereas all other samples where run under reduced pressure, 0.2 bar. The large difference in conversion between microemulsion 4 (atmospheric pressure) and microemulsion 5 (reduced pressure) indicates that in the former the conversion was restricted due to accumulation of water.

Figure 4 shows the conversion of fatty acid as a function of reaction time in the microemulsions based on the product as surfactant. As shown in the figure, the conversion increases with increasing amount of enzyme. After 24 h reaction time, the conversion in the system with the higher amount of enzyme was 77%, whereas it was 35% in the system with the lower amount of enzyme. Evidently, reaction kinetics determines the yield.

Product separation. When formulating a reaction system, separation of the product from the substrate must be taken into consideration. One way to solve this separation problem could be if the reaction system shows a separate surfactant phase. However, no separate surfactant phase was found in the systems that are presented in this paper. Nevertheless, it was possible to remove remaining fatty acid by means of washing with a sodium bicarbonate solution. After 24 h reaction time, sample 5 was washed four times, 200 mL each time, with a 2% bicarbonate solution. The amount of fatty acid was reduced more than 90%.

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[Received May 9, 1995; accepted September 12, 1996]