Synthesis and interfacial properties of sophorolipid derivatives

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Abstract

Biosurfactants made by fermentation from renewable resources provide “environmental friendly” processes and products. A natural sophorolipid mixture was produced by the yeast Candida bombicola when cultured on glucose and oleic acid. The sophorolipid mixture was chemically modified to form the corresponding sophorolipid alkyl (methyl, ethyl, propyl, and butyl) esters by reaction with the corresponding sodium alkoxides. Interfacial properties of these surfactants, such as surface tension reduction, aggregation, and adsorption, were systematically studied. It was found that the critical micelle concentration of sophorolipid esters decreases to about 1/2 per additional one CH2 group to the alkyl ester moiety. Interestingly, these surfactants were found to adsorb strongly on alumina but weakly on silica. They have properties that make them attractive candidates for uses in detergents, cosmetics, soil remediation, and enhanced oil recovery.

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1. Introduction

Biosurfactants are promising natural surfactants that offer several advantages over chemically synthesized surfactants, such as lower toxicity, biodegradability, and ecological acceptability [1]. Sophorolipids (SLs), a prominent member of the biosurfactant family, are microbial, extracellular, surface-active glycolipids. They are produced by cells of Candida bombicola when grown on carbohydrates, fatty acids, hydrocarbons or their mixtures [2–5]. These molecules have a disaccharide sophorose unit linked by a glycosidic bond to the hydroxyl group at the penultimate carbon of monounsaturated C18 (oleic) acid [6]. Sophorolipids, first described by Gorin et al. [2], exist as a mixture of eight major components (lactone and acid forms) with varying degrees of acetylation at the 6′ and 6′′ positions of the sophorose moiety (Fig. 1). Sophorolipids and their derivatives have great potential for applications in detergents, cosmetics, therapeutics and personal care products [7–10].

Attempts have been made for in vivo modification of sophorolipid structure during biosynthesis. Selective feeding of lipophilic substrates such as alkanes and fatty acids during fermentation effect changes in the sophorolipid structure [11]. Although this approach is promising, so far it has produced changes only in a small fraction of the sophorolipids produced and those changes have been limited to modifications in the lipid structure. An alternate approach is to use in vitro enzymatic methods for structural modifications of sophorolipids after their microbial synthesis. Chemical- and enzyme-catalyzed modifications of a natural sophorolipid mixture have been reported in the past [12,13]. The synthesis of alkyl esters, their regioselective diacylation at 6′ and 6′′ positions [12], and polymerization of a 6-O-acryloyl derivative were reported [14]. Enzyme-catalyzed decacylation of a sophorolactone (Fig. 1, R = Ac) in a biphasic medium using an acetylesterase and the conversion of sophorolipids into a glucose lipid using several glycosidases were also
Fig. 1. Structure of lactonic and acidic forms of sophorolipid mixture produced by C. bombicola.

Fig. 2. Diagram showing procedure for preparation of sophorolipid alkyl esters.

Glycolipid mixtures studied using either culture supernatants or isolates from the culture broth or cell fractions have been shown to cause substantial reductions in the surface tension [18–21]. By using the aqueous fermentation broth of Torulopsis apecola, the surface tension was lowered to 26 mN/m. However, to the best of our knowledge, systematic studies to evaluate the effects of structural variations in SLs on their surface-active properties have not been reported.

The purpose of this investigation was to understand relationships between the alkyl ester chain length, attached to the carboxyl end of the sophorolipid lipid moiety, and their interfacial properties. For this purpose, five different sophorolipid derivatives were prepared by treating a natural sophorolipid mixture with different sodium alkoxides (Fig. 2). The structure of modified sophorolipid derivatives was confirmed by mass, proton and carbon NMR spectra. Surface tension, adsorption and fluorescence spectroscopic studies were conducted to determine the colloidal and interfacial characteristics of these modified biosurfactants.

2. Experimental

2.1. Materials

2.1.1. Chemicals

All chemicals used for this investigation were of analytical grade and were used as received unless otherwise noted. The sophorolipid mixture was synthesized by fermentation of C. bombicola with glucose/oleic acid mixtures following a published procedure [3, 6]. HPLC grade methyl alcohol, ethyl alcohol, propyl alcohol, butyl alcohol, hexyl alcohol, silica gel, and sodium were obtained from Aldrich. All alcohols were distilled and stored over activated 4 Å molecular sieves prior to use. Chloroform/methanol mixtures (Pharmco) were used as eluent for purification of compounds.

2.1.2. Solids

Alumina AKP-50 obtained from Sumitomo had a mean diameter of 0.2 μm. The BET specific surface area measured using nitrogen with a Quantasorb system was 10.8 m²/g and the isoelectric point (iep) was 8.9. Silica obtained from Geltech had a mean diameter of 0.1 μm, specific surface area of 12.21 m²/g, and an iep around 2.
2.2.4. Adsorption studies

Adsorption experiments were conducted in capped 20 ml vials. A given amount of the solid sample was mixed with 10 ml of triple distilled water for 2 h. The pH was adjusted as desired and then 10 ml of the surfactant solution was added and the samples were equilibrated for 16 h. The surfactant concentrations after adsorption were determined by measuring the total organic carbon in the sample using a Beckman microbalance (Model LM 600). The entire assembly was kept in a draft-free enclosure at a temperature of 45 ± 1°C. For each measurement, the sensor was left in contact with the surfactant solution for 30 min to allow equilibration.

2.2.5. General procedure for the preparation of sodium alkoxydes

Sodium alkoxydes solutions (0.2N) were made by the slow addition of sodium metal to different alcohols with stirring at −5°C (ice/sodium chloride) under nitrogen atmosphere.

2.2.6. Procedure for the preparation of sophorolipid alkyl esters

Sodium alkoxide solutions (0.2N) were made by the slow addition of sodium metal to different alcohols with stirring at −5°C (ice/sodium chloride) under nitrogen atmosphere.

3. Results and discussion

The preparation and structural elucidation (using 1H and 13C NMR, DEPT, 1H-1H COSY, HETCOR, infrared spectroscopy and mass spectroscopy) of the sophorolipid methyl ester (2), sophorolipid ethyl ester (3), sophorolipid butyl ester (5), and sophorolipid hexyl ester (6) followed exactly as was previously reported [12].

The sophorolipid propyl ester (4) was prepared by the treatment of the natural sophorolipid mixture (2 g) with 0.2N sodium propoxide (25 ml) at room temperature for 3 h. The reaction mixture was acidified (pH 4) with glacial acetic acid and the solvent removed under reduced pressure. The residue was then subjected to silica gel column chromatography using chloroform/methanol (95:5, v/v) as eluent to give the corresponding pure alkyl esters.
to the ethyl ester (I) reported earlier [12], no other significant change in the $^{13}$C NMR spectrum of product 4 was found. Thus, these spectral analyses confirmed that the product formed was indeed structure 4.

Interfacial tension lowering and micellization in solution are the most fundamental characteristics of a surfactant. The surface tension of a series of sophorolipid esters were measured at 45°C and the results are shown in Fig. 3. As the chain length of the $n$-alkyl ester moiety increases, the critical micelle concentration (cmc) of the surfactants decreases.

The relationship between the cmc of a homologous series of surfactants and the carbon chain length (straight-chain), $n_c$, is usually expressed as:

$$\log(\text{cmc}) = A - Bn_c$$  \hspace{1cm} (1)

where $A$ and $B$ are constants specific to the homologous series and $n$ is the number of carbons on the hydrophobic chain of the surfactant. It has been reported that the cmc of alkyl-$\beta$-D-glucoside and alkyl-$\beta$-D-maltoside decreased by 1/3 for each additional CH$_2$ group [23]. The results of this investigation showed the cmc for methyl, ethyl, and propyl esters of sophorolipids decreased by about 1/2 per additional CH$_2$ group (Fig. 4). This is probably due to the fact that the added CH$_2$ group is close to the hydrophilic ester group. This
ester perturbs the hydrophobic character and the ability of the lipid moiety to pack into a micelle. Thus, the effect of additional CH$_2$ groups that are close and on the oxygen side to the ester may have a greater impact on cmc than CH$_2$ groups along a straight lipid moiety.

The minimum surface tension also decreases with increases in chain length. This can be explained by the increase of hydrophobicity of surfactants. As the carbon chain length increases, the surfactants become more surface active.

The adsorption density and the packing state of surfactants at the air/liquid interface can be obtained by analyzing the results in Fig. 3. Based on Gibbs adsorption equation, for dilute nonionic surfactants:

$$\Gamma_{\text{max}} = -\frac{1}{RT}\lim_{C \to \text{cmc}} \left( \frac{d\gamma}{d\ln C} \right)$$

(2)

Where $\gamma$ is the surface tension, $C$ the surfactant concentration, $R$ the gas constant, $T$ the absolute temperature and $N$ the Avogadro’s constant.

Eq. (2) shows that the surface area per molecule is a function of $\frac{d\gamma}{d\ln C}$, the slope of the surface tension isotherm close to cmc. The slopes are almost identical for methyl, ethyl, and propyl esters, suggesting that the surface adsorption density at cmc as well as the packing of these surfactants at the air/liquid interface is the same. The average adsorption density is $2.07 \times 10^{-6}$ mol/m$^2$ and area per molecule is 80 Å$^2$. The area per molecule for these surfactants is larger than those of other surfactants with similar structure such as sugar-based n-dodecyl-β-D-maltoside, which is $\sim 50$ Å$^2$ [24].

Surfactant micellization was also studied by the fluorescence spectroscopy technique. In fluorescence spectroscopy the ratio of relative intensities of the third peak (382 nm) to the first peak (372 nm) $I_3/I_1$ on a pyrene emission spectrum shows the greatest solvent dependency. This ratio decreases as the polarity increases and can be used to estimate the solvent polarity of an unknown environment in which the pyrene probe is situated. It can also be used to elucidate the surfactant micellization process due to the occupation of the probe inside the hydrophobic interior of micelles. The pyrene is incorporated exclusively within the micelle or the hydrocarbon environment during its excited lifetimes [25]. The polarity parameters of pyrene are shown in Fig. 5 as a function of sophorolipid ester concentration. At lower concentrations, the value of $I_3/I_1$ ratio corresponds to that of water ($\sim 0.58$). At certain concentration, this ratio decreases as the polarity increases and can be used to estimate the solvent polarity of an unknown environment in which the pyrene probe is situated. It can also be used to elucidate the surfactant micellization process due to the occupation of the probe inside the hydrophobic interior of micelles. The pyrene is incorporated exclusively within the micelle or the hydrocarbon environment during its excited lifetimes [25].
of micelles at this concentration. The cmc of sophorolipid methyl, ethyl, and propyl esters obtained from fluorescence tests are in good agreement with those obtained from surface tension measurements.

Adsorption of surfactants at solid/liquid interfaces is also a fundamental property of surfactants. Adsorption isotherms at neutral pH and 45°C of the sophorolipid methyl ester on two important hydrophilic solids, alumina and silica, are shown in Fig. 6. The surfactant is found to adsorb well on alumina with a sharp rise in the slope suggesting lateral interactions (hemimicelles). In contrast, adsorption on silica was much less without any hemimicellar type association. This behavior is same as that of the sugar-based n-dodecylβ-D-maltoside [24]. Polysaccharide polymers such as dextrin and starch also behave in a similar manner. In contrast to the above, another group of nonionic surfactants, the ethoxylated surfactants, adsorbs on silica but not on alumina [26]. Thus, the interfacial properties of sophorolipid esters and ethoxylated surfactants can be very different. It has been proposed that in the case of sugar-based n-dodecylβ-D-maltoside, hydrogen bonding between surfactant hydroxyl groups and the hydroxyl species on the alumina surface is the driving force for their adsorption on alumina [27]. Similarly, sophorolipids contain a sugar head group that appears to develop similar interactions with alumina as was found with n-dodecylβ-D-maltoside. The unique behavior of these surfactants may be exploited for selective adsorption phenomena to surfaces where hydrogen bonding is the dominant driving force.

Examination of the surfactant adsorption isotherm on alumina shows that adsorption occurs in three stages. In the first stage, the surfactant is believed to adsorb individually and sparsely through hydrogen bonding on the surface and chain–chain interaction is not significant. As mentioned earlier, a sharp increase in the adsorption density occurs in the second stage. This is explained by the association of the surfactants into hemimicelles due to chain–chain interactions [28,29]. The adsorption isotherm reaches a plateau region at the onset of the third stage. The inflection point between stages II and III corresponds to the cmc of the surfactant.

The influence of the sophorolipid n-alkyl ester chain length on its adsorption behavior was studied. The adsorption isotherms of the methyl, ethyl, propyl, and butyl sophorolipid esters were measured and the results are shown in Fig. 7. All the isotherms, except that of the butyl ester, are similar in shape with the same maximum adsorption densities. In the plateau region, the adsorption density is about $3.8 \times 10^{-6}$ mol/m² and the surface area per molecule adsorbed was $44 \text{Å}^2$. In comparison, the surface area per molecule at the solution/air interface is $80 \text{Å}^2$. Thus, these results indicate that in the plateau region, the methyl, ethyl, and propyl sophorolipid esters adsorb and self-assemble at the aluminum surface to form a bilayer. Indeed, such behavior is commonly found for surfactants adsorbed onto hydrophobic solids. Interestingly, the adsorption isotherm of the sophorolipid butyl ester is different from that of its shorter chain ester analogues. The sharp increase in the adsorption density that was found in region II for the methyl, ethyl, and propyl sophorolipid esters was not observed for the corresponding butyl ester. Furthermore, the maximum adsorption density of butyl ester is lower than those of the shorter chain esters studied.
4. Summary and conclusions

The chain length of the \( n \)-alkyl group (methyl, ethyl, propyl, butyl, hexyl) esterified to the sophorolipid fatty acid was varied. The effect of the \( n \)-alkyl ester chain length on the interfacial properties of the corresponding sophorolipid analogues was studied. The cmc and minimum surface tension have an inverse relationship with the alkyl ester chain length. The cmc decreased to 1/2 per additional CH2 group for the methyl, ethyl, and propyl series of chain lengths. These results were confirmed by fluorescence spectroscopy.

Adsorption of sophorolipid alkyl esters on hydrophilic solids was also studied to explore the type of lateral associations. These surfactants were found to adsorb on alumina but much less on silica. This adsorption behavior on hydrophilic solids is similar to that of sugar-based nonionic surfactants and unlike that of nonionic ethoxylated surfactants. Hydrogen bonding is proposed to be the primary driving force for adsorption of the sophorolipids on alumina. Increase in the \( n \)-alkyl ester chain length of the sophorolipids causes a shift of the adsorption isotherms to lower concentrations. The magnitude of the shift corresponds to the change in cmc of these surfactants.

The adsorption isotherm of the sophorolipid methyl ester on alumina shows a typical three-stage adsorption of nonionic surfactants on hydrophilic solids. The maximum adsorption density suggests bilayer formation at solid/liquid interfaces at higher concentrations. This study suggests that desirable surface-active agents can be prepared by careful modulation of the hydrophilic/lipophilic balance in naturally occurring biomolecules.

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