Foaming Properties of Surfactin, a Lipopeptide Biosurfactant from *Bacillus subtilis*

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ABSTRACT: Foaming properties of surfactin were investigated and compared to those of sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA). Foams were formed by a bubbling technique. Evolution of the foam volume and the liquid in the foam was monitored with optical and conductimetric methods to characterize foam formation and stability. Excellent foaming properties of surfactin were shown by its higher ability to form and stabilize the foam at a concentration as low as 0.05 mg/mL, in comparison with SDS and BSA. Surfactin produced a foam with intermediate maximum density and stabilized the liquid in foam, as well as BSA.
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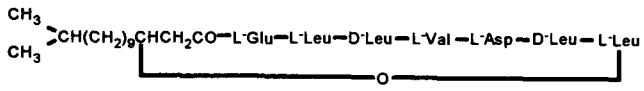
KEY WORDS: *Bacillus subtilis*, biosurfactant, foaming properties, lipopeptide, surfactin.

Surfactin is a cyclic lipopeptide produced by various strains of *Bacillus subtilis*. Its structure consists of a heptapeptide, which is closed at its extremities by ester and amide bonds with a β-hydroxy myristic acid (1) (Scheme 1):



SCHEME 1

A homologous series with lipidic chainlengths of 13, 14, and 15 carbon atoms (2,3), and isoforms—named [Val7], [Ile7], and [Ala4]surfactin and differing by the seventh or the fourth amino acid (4,5)—have been identified. Surfactin exhibits various properties, such as inhibition of fibrin clot formation, cytolytic and antibacterial activities (6), and ionophorous and sequestering properties (7), but it is especially known for its exceptional surface activity. It is one of the most potent biosurfactants, which are increasingly interesting because of their higher biodegradability as compared to synthetic surfactants (8). Despite its strong surface activity, surfactin functional properties, such as foaming, emulsifying, and wetting abilities, have not been evaluated. The investigation of foaming properties of surfactin appears particularly attractive. Indeed, the two main classes of foaming agents are small surfactant molecules, which are very effective in forming foams, and proteins, which are more appropriate to stabilize them. As surfactin contains both a lipidic chain, generally found in small surfactant molecules, and a peptidic chain, which is the basic constituent of proteins, its hybrid structure prompted us to evaluate its ability to form and stabilize foams.

EXPERIMENTAL PROCEDURES

Surfactin (surfactin with β-hydroxymyristic acid) was produced by fermentation of the *B. subtilis* strain S499 in optimized culture media as previously described (9). Its preparation included extraction (3), fractionation (4), and final purification by reversed-phase chromatography in a chromspher 5 μm C18 column (1 × 25 cm; Chrompack, Middelburg, The Netherlands), flow rate, 4 mL/min, mobile phase, acetonitrile/H_2O/trifluoroacetic acid 0.05%, 80% by vol; and detection, ultraviolet at 214 nm. Its purity and structure were ascertained by amino acid analysis (3) and electrospray mass spectrometry (Hbid, C., P. Jacques, H. Razafindralambo, G. Ghitti, R. Renzoni, M. Paquot, E. DePauw, A. Germain, and P. Thonart, manuscript in preparation). Bovine serum albumin (BSA) and sodium dodecyl sulfate (SDS) were from Sigma (St. Louis, MO) (purity 96–99%) and Merck (Darmstadt, Germany) (purity >98%), respectively, and were used without further purification. Foaming properties were analyzed by optical and conductimetric techniques, which allowed us to continuously measure the volume and the liquid fraction of the foam during and after its formation (10) under the following conditions: air flow rate, 20 mL/min; disk porosity diameter, 2 μm; column size, 20 × 2 cm; volume of sample, 8 mL; required foam volume, 35 mL; and analysis duration, 20 min. Samples (0.05–0.2 mg/mL) were dissolved in 5 mM Tris buffer at pH 8 prepared with milli-Q water (Millipore Corporation, Milford, MA). All measurements were carried out at 22°C. Each analysis was performed at least twice.

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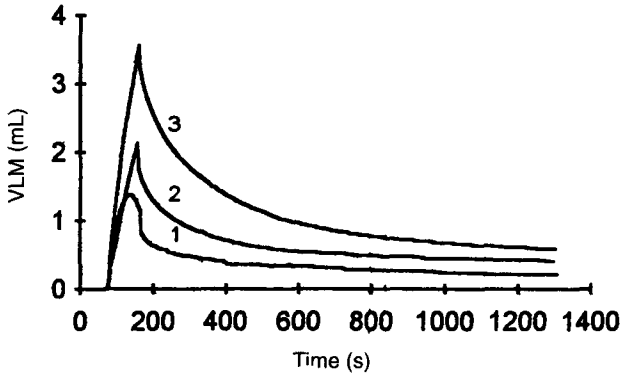

FIG. 1. Volume of liquid in foam (VLM) vs. time curves of surfactin: 1, 0.05 mg/mL; 2, 0.1 mg/mL; and 3, 0.2 mg/mL.

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the liquid quantity in surfactin foam during and after bubbling. The time necessary to form the required foam volume (35 mL) was similar (101 ± 5 s) for different concentrations (0.05–0.2 mg/mL), while the maximum quantity of liquid in the foam increased with the concentration.

At 0.05 mg/mL, SDS and BSA form unstable foams during the foaming process and cannot produce the required foam volume. These results demonstrate the effectiveness of surfactin as a foaming agent based on the quantity necessary to achieve required results. This is not surprising because of the higher surface activity of surfactin (6) as compared to SDS and BSA (11). Comparisons with SDS and BSA were done at higher concentrations. Various parameters that characterize foam formation and stability of SDS, BSA, and surfactin were calculated and are presented in Table 1.

Foaming capacity (FC) of surfactin is higher than that of SDS. However, its maximum foam density (MD) is inferior to that of SDS. This is generally related to the ability of low-molecular weight surfactants, like SDS, to adsorb at the interface and expand the liquid film rapidly. A lower FC with a higher MD for SDS indicates that the collapse of air bubbles occurred during foam formation. That is probably due to the weak mechanical strength of the SDS-adsorbed film. According to the residual foam volume after 20 min and the half-life time of liquid in foam (T1/2), surfactin foaming stability appears much higher than that of SDS.

Compared to BSA, the FC and MD of surfactin are higher, even for a concentration four times lower. According to T1/2, the aptitude of surfactin film to reduce the drainage rate is similar to that of BSA. This suggests good rheological properties of surfactin films, which are the main factors of foam stabilization by proteins (12). On the other hand, surfactin capacity to stabilize foam volume, shown by the residual foam after 20 min, is greater than that of BSA. This indicates the higher resistance of surfactin film to the coalescence of bubbles, in comparison with that of BSA. Moreover, thinner bubbles were observed for surfactin foam as compared to those of SDS and BSA.

TABLE 1
Characteristic Parameters of Foaming Properties of Surfactin, Sodium Dodecyl Sulfate (SDS), and Bovine Serum Albumin (BSA)

Amphiphilic molecules	Concentration (mg/mL)	FC[a]	MD[b]	RV[c] (%)	T1/2[d] (s)
Surfactin	0.05	0.98	0.03	34	57
	0.1	1.01	0.05	88	91
	0.2	1.12	0.10	94	141
SDS	0.1	0.80	0.13	0	76
BSA	0.2	0.94	0.03	65	147

[a]FC, maximum volume of foam/volume of gas injected.
[b]MD, maximum liquid volume/maximum foam volume.
[c]RV, residual volume after 20 min.
[d]T1/2, half-life time of liquid in foam.

These results show the high performance of surfactin as a foaming agent compared to SDS and BSA, which are representative of the two main classes of amphiphilic molecules. Its excellent foaming capacity and stability arise essentially from its strong surface activity, and is probably also due to good mechanical and rheological properties of its surface-adsorbed film. Its hybrid and intermediate structure appears to be of great benefit for foaming properties. Accordingly, lipopeptidic structures could serve as a molecular model to develop effective foaming agents, which are of considerable interest for product formulations in detergent, cosmetic, and pharmaceutical applications.

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