Food Emulsions and Foams

Interfaces, Interactions and Stability

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Structure, Interfacial Properties, and Functional Qualities in Foams and Emulsions of Surfactin, a Lipopeptide from *Bacillus subtilis*

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1 Introduction

Surface-active agents are required for forming and stabilizing disperse systems such as foams and emulsions. They find applications[1–5] in an extremely wide variety of areas involving product formulation in food, cosmetics, road, pesticide, detergent, paper, and pharmaceutical industries, as well as enhanced oil recovery, transportation of heavy crude oil, and bioremediation.

In molecular terms, surface-active agents are amphiphilic compounds containing both hydrophilic and lipophilic parts.[6] Their efficiency in foaming and emulsifying is dependent on their amphiphilic structure. In general, two main types of surface-active agents can be distinguished: small surfactant molecules and amphiphilic macromolecules. Because of their small size and their simple amphiphilic structure composed of a polar head and a hydrophobic tail, small surfactant molecules diffuse and orient rapidly at fluid–fluid interfaces. They reduce efficiently the interfacial tension and promote disperse system formation.

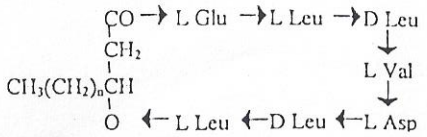

Figure 1 *Example of surfactin primary structure (n = 9, 10, or 11)*

On the other hand, amphiphilic macromolecules like proteins have a high molecular weight and a more complex multi-amphiphilic structure. They migrate less quickly to the interface, but they form a cohesive viscoelastic film via intermolecular interactions for greater long-term stability.[7,8] A perfect surface-active agent should combine the favourable features of proteins with those of the most effective small surfactant molecules.[6]

Bacillus subtilis surfactin is a typical compound which could satisfy this condition owing to its hybrid structure and intermediate size in comparison with small surfactant molecules and proteins (see Figure 1). Its structure is composed of a heptapeptide cycle closed by a β-hydroxyfatty acid that forms a lactone ring system.[9] In addition, as biosurfactant, surfactin exhibits several advantages such as biodegradability, low toxicity, and various possible structures, relative to chemically synthetized surfactants.[3,10,11]

The present paper reports a study on the effect of the hydrophobic character of the surfactin lipidic chain on the interfacial properties and functional qualities of this type of molecule in foam and emulsion systems. A comparison between foaming and emulsifying performances of surfactin variants is discussed. To acquire such information, three surfactin homologues (SuC13, SuC14 and SuC15) produced by the *Bacillus subtilis* S499 strain have been purified. Their interfacial properties at both air–water and oil–water interfaces have then been studied. Investigations include the characterization of the adsorption under dynamic conditions, and the evaluation of the foaming and emulsifying properties by studying the short-term destabilization rate of foams and emulsions.

2 Materials and Methods

Surfactins were produced by fermentation of the *B. subtilis* strain S499[12] and purified as previously described.[13] Primary structure and purity of the surfactin homologous series (>99%) were ascertained by analytical RP-HPLC (chromsphere-5 μm C18 column, 1 × 25 cm, Chrompack, Middelburg, Netherlands), amino acid analysis,[14] and electrospray mass spectrometry measurements using a Finnigan MAT 900 ST. Three surfactin homologues containing the β-hydroxyfatty acids of 13 (SuC13, MW: 1007), 14 (SuC14, MW: 1021) and 15 (SuC15, MW: 1035) carbon atoms were obtained. SuC13 and SuC15 have an iso-branched β-hydroxyfatty acid, while the SuC14 fatty acid is linear.

n-Dodecane was purchased from Sigma (purity >99%, St. Louis, MO) and

n-hexadecane from Merck (purity for analysis, Darmstadt, Germany). All other reagents were analytical grade. Milli-Q water was prepared by Millipore apparatus (Millipore Co., Milford, MA). All surfactin samples were dissolved in 5 mM tris buffer prepared with Milli-Q water and adjusted to pH 8.0–8.5. All measurements were carried out at 20–22 °C. Each analysis was performed at least twice.

Adsorption kinetics at the air–water (A–W) and oil–water (O–W) interfaces were monitored continuously by following the decrease in surface or interfacial tension. The measurements were carried out with a drop volume tensiometer (TVT1, Lauda) in the dynamic mode.

Dynamic surface or interfacial tension *versus* time plots, $\gamma = f(t)$, were described by the relaxation equation used by several authors:[15,16]

$$\gamma_t = \gamma_m + (\gamma_0 - \gamma_m)/[1 + (t/t^*)^n]. \quad (1)$$

Here γ_0 is the surface or interfacial tension of the pure solvent, γ_t is the surface or interfacial tension at time t, γ_m is the surface or interfacial tension at meso-equilibrium, t^* is the half-time for reaching γ_m, and n is a dimensionless constant. Parameters n, t^*, and γ_m were estimated by computer fitting of the measured dynamic surface or interfacial tension data using Sigma-plot software (Jandel, Germany). By differentiating equation (1) with respect to t and substituting t for t^*, the maximum reduction rate v_{max} of γ is obtained to be

$$v_{max} = \frac{n(\gamma_0 - \gamma_m)}{4t^*} = -(d\gamma_t/dt)_{max}. \quad (2)$$

Creaming and flocculation kinetics were determined using a conductimetric method developed by Guéguen *et al.*[17] Surfactin solutions at 0.1 mg ml^{-1} (7.5 ml) and *n*-hexadecane (4.5 ml) were poured into a cylinder containing two electrodes at the base. The conductivity of the aqueous phase was measured before emulsification, and then the emulsion was formed using ultrasonic treatment (15 s, 35 W, 23 kHz). The volume of the aqueous phase was continuously monitored by conductivity measurement. The rate of destabilization of the emulsion (k_1) was measured from ϕ to $\phi + 0.1$ from ϕ *versus* time plots, where ϕ is the volume fraction of dispersed oil in the emulsion, calculated from

$$\phi = 1 - [7.5/4.5 \times (1 - C_{tc}/C_{sol})], \quad (3)$$

where C_{sol} is the conductivity of the aqueous solution before emulsification and C_{tc} is the conductivity of the emulsion at time t.

3 Results and Discussion

Adsorption at Air–Water and *n*-Dodecane–Water Interfaces

Figure 2 shows dynamic surface tension curves of SuC13, SuC14 and SuC15 at

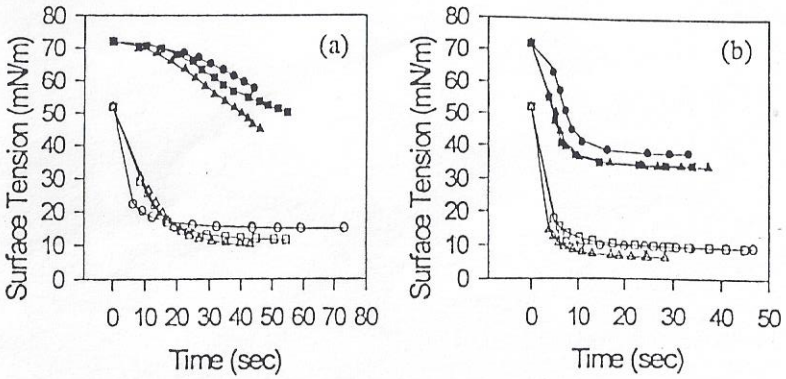

Figure 2 *Curves $\gamma = f(t)$ of surfactins: (a) at 2.0 × 10^{-8} mole cm^{-3}, (b) at 7.0 × 10^{-8} mol cm^{-3}, at A–W interface (filled symbols) and O–W interface (open symbols) for homologues C13 (●,○), C14 (■,□) and C15 (▲,△)*

air–water (A–W) and *n*-dodecane–water (O–W) interfaces at two concentrations, a lower one of 2.0 × 10^{-8} mol cm^{-3} and a higher one at 7.0 × 10^{-8} mol cm^{-3}. Generally speaking, the surfactins reduce the dynamic surface tension. These results reveal that surfactins adsorb at the A–W or O–W interfaces and present surface-active properties at these two interfaces. This general property is related to the amphiphilic character of surfactins due to the presence of a hydrophobic part consisting of a long-chain fatty acid and some lipophilic amino acids, and a hydrophilic part composed of several amino acid residues.

At lower concentrations, the effect of lipid chain hydrophobic character on the surface tension is different at the A–W and O–W interfaces. At the A–W interface, the higher the lipid chain hydrophobicity the faster the reduction of γ, while at the O–W interface, the SuC13 variant is more efficient in kinetic terms than SuC14 and SuC15. The difference between the homologous molecules could be attributed to a difference in the area occupied per molecule, which has a consequence on the surface tension. Indeed, at the A–W interface, it has been shown[18] that the increasing hydrophobicity of a tail group enhances the molecular area occupied.

At higher concentrations, differences between the three homologous surfactins are negligible at A–W as well as at O–W interfaces. This suggests that the homologous molecules occupy the same interfacial area, with perpendicular orientation of the lipid chain at either A–W or O–W interfaces at high concentration. At high and low concentrations, the decrease of surface tension is greater at the O–W interface than at the A–W interface.

From the curves at Figure 2, the kinetic parameters γ_m, n, t^* and v_{max} have been calculated. The values are listed in Table 1. As already noted from curves in Figure 2, the effect of lipid chain hydrophobicity on the surfactin kinetic behaviour is different for the two interfaces except for the value of the parameter γ_m. At low and high concentrations, γ_m decreases with strengthening of lipid

Table 1 *Kinetic parameters of curves $\gamma = f(t)$ of surfactin homologues at air–water (A–W) and n-dodecane–water (O–W) interfaces. Surfactin solutions were prepared in 5 mM tris buffer at pH 8.0*

Surfactin	Conc./ mol cm^{-3}	γ_m/mN m^{-1}		n		t*/s		v_{max}/ mN m^{-1} s^{-1}	
		A–W	O–W	A–W	O–W	A–W	O–W	A–W	O–W
C13	2.0	47.24	14.50	1.73	1.27	48.58	2.30	0.22	5.18
	7.0	37.85	8.47	3.88	0.48	6.48	n.d.[a]	5.10	n.d.[a]
C14	2.0	44.40	11.03	3.00	2.15	36.09	7.89	0.57	2.79
	7.0	34.45	7.59	3.16	1.49	4.12	1.87	7.18	8.85
C15	2.0	36.33	8.15	3.01	2.07	38.28	9.25	0.80	2.45
	7.0	33.84	6.13	2.71	1.62	4.40	1.48	5.86	12.55

[a]n.d.: not determined. The tension for SuC13 decreases so quickly at the O–W interface that curve fitting cannot be realized.

chain hydrophobic character for both interfaces. Increased hydrophobicity increases the degree of interaction between the chains which strengthens the surfactin effect on the dynamic surface free energy.

At high concentration, the n value dependence on lipidic tail hydrophobicity is of opposite sign at A–W and O–W interfaces. The parameter n decreases with increasing chain hydrophobic character at the A–W interface whereas it increases at the O–W interface. According to Gao and Rosen,[19] the parameter n is related to the difference between the adsorption rate and the desorption rate. The closer the n value is to zero, the more the adsorption is near to equilibrium, *i.e.*, the adsorption rate is approaching the desorption rate. Based on this interpretation, increasing chain hydrophobicity at high concentration allows the equilibrium state to be reached more quickly at the A–W interface, while it slows down approach to the equilibrium state at the O–W interface. At low concentration, SuC13 has a lower n value than SuC14 and SuC15 at A–W and O–W interfaces. Thus, weaker hydrophobicity at low concentration allows the equilibrium state to be reached more rapidly whatever the interface type.

According to v_{max} values at low concentration, which represents the maximum rate of reducing γ at t^*, the increasing chain hydrophobicity leads to a decrease in the adsorption of surfactin at the O–W interface and to an increase at the A–W interface. The ideal chain hydrophobicity for fast reduction of γ at low concentration depends on the type of interface. SuC15 is more efficient in kinetic terms at the A–W interface while SuC13 is better at the O–W interface.

At high concentration, the more efficient in kinetic terms at the A–W interface is now SuC14 according to the values of t^* and v_{max}. At the O–W interface, it seems that SuC13 decreases γ more quickly than SuC14 and SuC15: the final γ value is reached after 5 seconds with SuC13, while it is obtained after 10–15 seconds with SuC14 and SuC15 (Figure 2).

Overall, the kinetic parameters show that the surfactant adsorption is more efficient at the O–W interface than at the A–W interface. This means that

surfactins reduce the tension faster and lower, and reach more quickly the equilibrium adsorption state, when the apolar phase is liquid and not gaseous.

The global difference between the interfaces could be explained by a different lipid chain orientation and/or peptidic cycle disposition at the interface. Indeed, an apolar liquid phase is a better apolar solvent than air. According to Miller,[20] at a liquid–liquid interface there is protruding of protein parts into both adjacent liquid phases, while at an A–W interface the protruding occurs only into the water phase. Thus, the dodecane phase can exert an attraction on carbon chains of the lipid tail and on the hydrophobic amino acids residues of the peptidic ring. Consequently, the lipid chain of surfactin would be more vertically organized at the O–W interface than at the A–W interface, where the lipid tails are rather more inclined to interact laterally,[21] and the peptidic saddle[22] would be more likely to enter into the apolar phase. This could contribute to a more organized and compressed structure at the O–W interface, and so could explain the greater effect of surfactins on surface tension and on kinetic parameters at this interface.

The discrepancy between A–W and O–W interfaces with regard to the effect of increasing chain hydrophobicity could be explained on the basis of the above considerations. The variation of lipid chain hydrophobicity could influence the molecular disposition between polar and apolar phases, whatever the phase type. At the O–W interface the greater attraction of the apolar phase would tend to exert an additional effect on molecular disposition at the interface. These two effects could contribute to a more structured molecular organization at the O–W interface with increasing hydrophobicity of the lipid chain at the two concentrations investigated, which could explain that a molecule needs a higher energy to compress the film in order to create a space large enough to adsorb[23] and consequently the existence of an adsorption energy barrier for high hydrophobicity at this interface. At the A–W interface, a higher concentration is needed to overcome the adsorption energy barrier with a surfactin of high chain hydrophobicity. This could be due to the fact that the molecular organization is looser at this interface, and that, consequently, a higher concentration is required to allow the highly hydrophobic chains to interact with each other and to interfere with the adsorption of other molecules.

Foaming and Emulsifying Performances

Destabilization mechanisms encountered in foams and emulsions are rather similar. The physical changes that may occur in emulsions are creaming, flocculation and coalescence. In foams, the process of drainage of continuous phase from thin films between the bubbles effectively takes the place of creaming and flocculation in emulsions. An additional instability process in foams is Ostwald ripening.[24,25] Creaming, flocculation and drainage are destabilization phenomena that usually occur over a short time in comparison to coalescence.

Table 2 presents the effect of increasing lipid chain hydrophobicity on short-term foaming and emulsifying stability. The effect of increasing chain hydrophobicity on the destabilization rate is different for foams and emulsions. In

Table 2 *Destabilization rate of surfactin foam and emulsion. LP1 is the initial drainage rate in foams and* k_1 *is the creaming/flocculation rate in emulsions*

Surfactin	*Foams*[a] *LP1/ml min*$^{-1}$	*Emulsions* $k_1/10^{-3}$ *min*$^{-1}$
C13	2.70 ± 0.24	<0.1
C14	2.26 ± 0.03	0.675 ± 0.066
C15	2.50 ± 0.00	0.781 ± 0.020

[a]Results from Razafindralambo *et al.*[13]

foams, SuC14 exhibits the highest stability with respect to liquid drainage according to the initial drainage rate (LP1). In emulsions, SuC13 develops the best resistance to creaming/flocculation phenomena according to the creaming/flocculation rate (k_1). Thus, the required molecular structure under the investigated conditions to stabilize foams is different from that for emulsions. In foams, there exists an optimum lipid chain hydrophobicity providing surfactin with the maximum short-term stability, while, in emulsions, a low hydrophobicity of the lipid tail, in the C13–C15 surfactin series investigated, favours short-term stability.

To prevent liquid drainage in foams, the foaming agent must form an efficient viscoelastic film. It has already been demonstrated[26] that too short a lipid chain produces insufficient cohesiveness, whereas too great a length produces too much rigidity for good film elasticity. This is in agreement with results from Razafindralambo *et al.*[13] In emulsions, the extent of flocculation mainly depends on the repulsive forces between droplets. Our results can be explained by the fact that an increase of chain hydrophobicity would modify the peptidic ring disposition at the interface, which would in turn influence the steric and/or electrostatic repulsion between droplets. A conformational analysis at the O–W interface of the three homologues could be used to assess this assumption.

4 Conclusions

Under dynamic conditions, for example in the processes of foam or emulsion formation, the dynamics of adsorption play an important role in the stabilization mechanism.[20] In the first part of this paper, a kinetic study of three homologues of surfactin at the A–W and O–W interfaces has been undertaken. From the results, all of the surfactins appear efficient for reducing the interfacial tension rapidly at the two interface types, and so they can find applications in foams as well as in emulsion systems. It would even seem that surfactins are more efficient for promoting emulsion formation than foam formation.

The kinetic behaviour of surfactin homologues is different at A–W and O–W interfaces. In general, SuC13 is more efficient, in kinetic terms, in emulsion

systems, while a higher homologue is more adapted for foam systems. However, SuC15 allows a lower γ value to be reached at the two interfaces.

In the second part of this report, a discussion about the chain hydrophobicity effect on short-term stability of foams and emulsions is presented. It has been found that SuC13 develops the best short-term stability in emulsions, while SuC14 is more fitted to stabilize foams. From these results, it appears that there exists a correlation between the dynamic behaviour at high concentration and the short-term stability as regard to the chain length effect of surfactin. Conformational analysis at the interface of the three homologous surfactins is under investigation by our group in order to assess the role of molecular disposition at the interface on the surface functional properties.

It has been shown from theory[27,28] that the mechanical properties of the interfacial layer is also an important parameter in the stabilization mechanism. Experiments on surface rheological properties of surfactin monolayers would be thus of great interest.

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