

## Interactions of Surfactin with Membrane Models

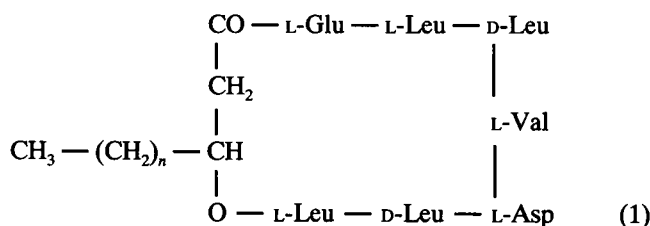
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**ABSTRACT** Surfactin, an acidic cyclic lipopeptide produced by strains of *Bacillus subtilis*, is a powerful biosurfactant possessing biological activities. Interactions of ionized surfactin (two negative charges) with lecithin vesicles have been monitored by changes in its CD spectra. These changes are more important in the presence of  $\text{Ca}^{2+}$  ions. We have studied the penetration of ionized surfactin into lipid monolayers. Using dimyristoyl phospholipids, the surfactin penetration is more important in DMPC than in DMPE monolayers and is greatly reduced in DMPA monolayers because of electrostatic repulsion. The surfactin penetration is lowered when the acyl chain length of the phospholipids increases. The exclusion pressure varies from  $40 \text{ mN m}^{-1}$  for DMPC to  $30 \text{ mN m}^{-1}$  for DPPC and  $18 \text{ mN m}^{-1}$  for egg lecithin. The presence of  $\text{Ca}^{2+}$  ions, which neutralize the charges of both surfactin and lipids in the subphase, leads to an important change of the penetration process that is enhanced in the case of acidic, but also of long chain (higher than  $\text{C}_{14}$ ) zwitterionic phospholipids (DPPC and lecithin). From compression isotherms of mixed surfactin/phospholipid monolayers, it appears that surfactin is completely miscible with phospholipids. The present study shows that surfactin penetrates spontaneously into lipid membranes by means of hydrophobic interactions. The insertion in the lipid membrane is accompanied by a conformation change of the peptide cycle.

### INTRODUCTION

Surfactin is an acidic cyclic lipopeptide produced by several strains of *Bacillus subtilis*. It is composed of a heptapeptide cycle closed by a  $\text{C}_{14-15}$   $\beta$  hydroxy fatty acid that forms a lactone ring system (Kakinuma et al., 1969).



This structure presents large similarities with those of iturins, another class of lipopeptides also produced by *B. subtilis* (Peypoux et al., 1978). From its structure, the amphiphilic nature of surfactin is obvious and explains that it is a very powerful biosurfactant, the surface properties of which have been described recently (Maget-Dana and Ptak, 1992). In addition to its biosurfactant character, surfactin exhibits interesting biological activities: it is an antibiotic substance also with antitumor activity (Kameda et al., 1974), it inhibits fibrin clot formation (Arima et al., 1968), lyses erythrocytes (Berheimer and Avigad, 1970), and has been shown recently to possess anti-HIV activity (Itokawa et al., 1994). Although the molecular mechanism of action of surfactin is still unknown, these biological properties are presumed to be the result of interactions with the membranes of target cells. In-

deed, it has been demonstrated that surfactin induces selective cationic channels in lipid bilayer membranes (Sheppard et al., 1991).

Because of its biosurfactant as well as its biological properties, surfactin gives rise to a growing interest from scientists and manufacturers (Georgiou et al., 1992). Several variants of surfactin have been isolated that differ by the peptide backbone (Peypoux et al., 1991; Baumgart et al., 1991; Oka et al., 1993; Peypoux et al., 1994; Itokawa et al., 1994). Today, laboratories are attempting to improve the surfactin production (Sheppard and Cooper, 1991), and genetic engineering is applied to this aim (Hiraoka et al., 1992; Morikawa et al., 1993). Moreover, the finding that surfactin is co-produced with iturin A by a same strain of *B. subtilis* (Sandrin et al., 1990) and exerts an important synergistic effect on the biological activities of iturin A (Thimon et al., 1992b; Maget-Dana et al., 1992) has renewed the interest on both lipopeptides.

Here we report on the interaction of surfactin with membrane model systems. Lipid vesicles are used to study the conformational changes of surfactin interacting with membranes. The monolayer technique is used to investigate the lipid specificity of the insertion of surfactin in the membrane. This technique is particularly suitable to mimic the phenomena occurring at the cell membrane/extracellular medium interface. The isotherm curves of spread surfactin/lipid mixed monolayers have also been recorded to characterize the molecular interactions and the mixing behavior of these compounds.

### MATERIALS AND METHODS

#### Materials

Surfactin was a gift of F. Peypoux (Laboratoire de Biochimie Microbienne, Lyon, France). Phospholipids:  $\text{L-}\alpha$ -dimyristoyl phosphatidylcholine (DMPC),  $\text{L-}\alpha$ -dimyristoyl phosphatidylethanolamine (DMPE),  $\text{L-}\alpha$ -dimyristoyl phosphatidic acid (DMPA),  $\text{L-}\alpha$ -dipalmitoyl phosphatidylcholine

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line (DPPC), and beef brain phosphatidylserine (PS) were purchased from Sigma Chemical Co. (St. Louis, MO). Egg lecithin was prepared according to Singleton et al. (1965), and cholesterol from Prolabo (Paris, France) was recrystallized twice from ethanol. Spreading solvents, hexafluoroisopropanol and dioxane, were from Merck (Darmstadt, Germany). Pure water was obtained from a Millipore (Milli Q) apparatus (Bedford, MA). Unilamellar lipid (egg lecithin) vesicles were prepared under sonication in a bath by a modified injection method (Kremer et al., 1977). Their mean diameter was about 50 nm as shown by electron microscopy after negative staining.

### Circular dichroism spectroscopy

Circular dichroism (CD) spectra were recorded at 20°C on a Jobin-Yvon IV autodichrograph using 1-mm path length cells. Lipid vesicles were added to the surfactin solution to obtain the required lipid/surfactin ratio. Absorption spectra of the same solutions were recorded with a Perkin-Elmer Lambda 15 spectrometer (Norwalk, CT). Absorbance values are always less than 1 in the wavelength range of the CD experiments. The contribution of the lipid vesicles was subtracted. The resulting optical activities are reported as the ellipticity per peptide residue,  $\theta_{\text{res}}$  (unit: deg cm<sup>2</sup> dmol<sup>-1</sup>).

### Monolayer measurements

Adsorption experiments were performed at constant area (19.6 cm<sup>2</sup>) in a thermostatically ( $T = 20 \pm 0.2^\circ\text{C}$ ) glass vessel (volume 10–15 ml) containing Tris 30 mM, pH 8.5 as a subphase. The lipid monolayers were spread at the air/water interface from a hexafluoroisopropanol/dioxane (5:1 v/v) mixture (except for PS, for which a chloroform/methanol 1:1 mixture was used) to give the desired initial surface pressure  $\pi_i$ . The vessel was equipped with a short vertical tube ( $\phi = 3$  mm) through which the surfactin solution was injected beneath the lipid monolayer. The surface tension was measured by means of a Wilhelmy platinum plate hung to a Q11 balance (Schenk SA, Darmstadt, Germany). The data were collected into a M24 Olivetti computer and directly converted into surface pressure values  $\pi = \gamma_0 - \gamma$  ( $\gamma_0$ , surface tension of the pure aqueous subphase). The accuracy of measurements was  $\pm 0.5$  mN m<sup>-1</sup>.

Mixed monolayer experiments were carried out by using a Langmuir film balance system conceived in our laboratory by A. Sanson.<sup>1</sup> The teflon trough rested on an antivibration plate and was enclosed in a plexiglass box. The temperature of the trough was maintained at  $20 \pm 0.2^\circ\text{C}$  by external circulating water. The surfactin and lipid solutions were mixed before spreading at the air/water interface with a 25- or 50- $\mu\text{l}$  Hamilton microsyringe. The compression isotherm curves were recorded at a compression rate comprised between 0.05 and 0.1 nm<sup>2</sup> mol<sup>-1</sup> min<sup>-1</sup> by means of a step-by-step motor. The reproducibility of molecular areas was  $\pm 2\%$ . The information was stored into an Apple II computer.

## RESULTS

### Surfactin interaction with lipid vesicles as monitored by CD measurements

CD spectra of surfactin have not been reported previously. The main features of the ionized surfactin spectra are a negative band centered at 192 nm, a small positive band centered at  $\approx 207$  nm, and another small negative band centered at  $\approx 224$  nm. These features are indicative of turns (Johnson, 1990). The presence of Ca<sup>2+</sup> ions induces important changes in the CD spectra. As seen in Fig. 1 *a*, the negative band at 192 nm becomes a positive minimum, the positive band at

203 nm and the negative one at 224 nm are enhanced and slightly blue-shifted. These features indicate a change in the conformation of the peptide cycle that may adopt another type of turns upon Ca<sup>2+</sup> binding.

The addition of egg lecithin vesicles to a surfactin solution in the absence of Ca<sup>2+</sup> provokes the disappearance of the positive band at 207 nm. In the presence of Ca<sup>2+</sup> ions, an increase of the ellipticity with a slight blue-shift of the minimum (from 221 to 218 nm) is observed. These modifications of the CD spectra indicate changes in the conformation of surfactin resulting from interactions with lipid vesicles.

### Surfactin penetration in phospholipid monolayers

At the air/water interface, both L-Glu<sup>1</sup> and L-Asp<sup>5</sup> residues are fully ionized at pH 8.5 (Maget-Dana and Ptak, 1992). The effect of surfactin injection beneath a phospholipid monolayer is shown in Fig. 2. We observe an instantaneous increase of the surface pressure of the monolayer, indicating that surfactin penetrates readily into the monolayer. In fact, agents interacting solely with the lipid head groups without penetration into the monolayer were shown not to affect the surface pressure (Demel et al., 1973). In Fig. 2 *b*, the surface pressure increase,  $\Delta\pi$ , of a DMPC monolayer at a given initial pressure is plotted as a function of the surfactin concentration,  $C$ , in the subphase. The magnitude of  $\Delta\pi$  varies inversely to the initial pressure of the lipid monolayer,  $\pi_i$ . By plotting, for a given surfactin concentration,  $\Delta\pi$  versus  $\pi_i$ , we can determine the so-called exclusion pressure (Fig. 3). This exclusion pressure is the initial surface pressure of a monolayer above which the lipopeptide is no longer able to penetrate the lipid monolayer and to cause a surface pressure increase (Bougis et al., 1981). This parameter, which is related to the packing of lipids, is a quantitative measure of the penetration power of a membrane effector, and then, of the affinity between the effector and the lipid constituents of the monolayer.

To investigate the specificity of surfactin with respect to phospholipids, we have studied in a comparative way the surfactin penetration into monolayers containing phospholipids of various polar heads and chain lengths.

#### Effect of polar head

Fig. 3 *a* compares the penetration power of surfactin ( $5 \times 10^{-7}$  M in the subphase) into some dimyristoyl phospholipids. In zwitterionic lipid monolayers (DMPC and DMPE),  $\Delta\pi$  is maximum when the initial pressure of the monolayer is around 5 mN m<sup>-1</sup>.  $\Delta\pi$  then reaches  $\approx 24$  mN m<sup>-1</sup> for DMPC and  $\approx 22$  mN m<sup>-1</sup> for DMPE monolayers.  $\Delta\pi$  decreases linearly when the lipid packing increases and in both cases we find the same exclusion pressure value ( $\approx 40$  mN m<sup>-1</sup>) which is probably the maximum pressure value attainable in the monolayer (this value corresponds approximately to the collapse pressure of DMPE).

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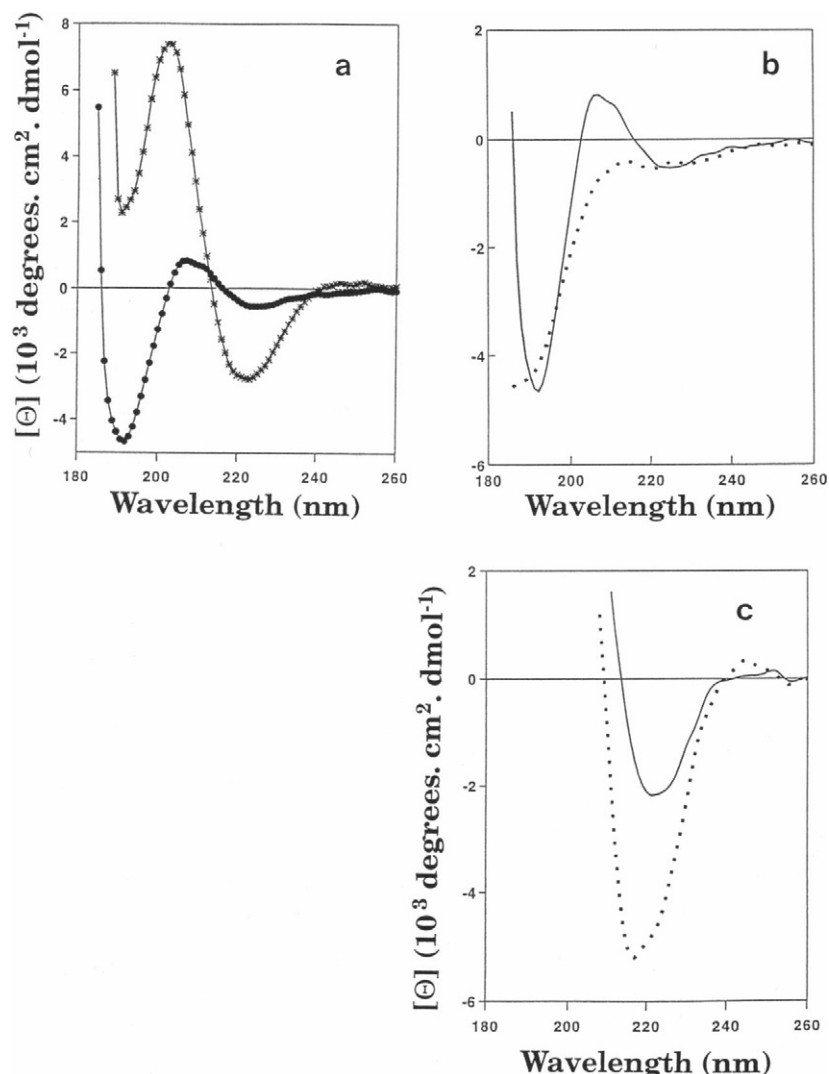


FIGURE 1 Circular dichroic spectra of surfactin. (a) (●) Surfactin  $1.1 \times 10^{-4}$  M and (\*) in the presence of  $2 \times 10^{-3}$  M  $\text{CaCl}_2$ . (b) Surfactin  $1.1 \times 10^{-4}$  M and (· · · · ·) in the presence of egg lecithin vesicles ( $5 \times 10^{-4}$  M phospholipid; phospholipid to surfactin molar ratio  $R = 5$ ). (c) Surfactin  $5.5 \times 10^{-5}$  M +  $2 \times 10^{-3}$  M  $\text{CaCl}_2$  and (· · · · ·) in the presence of lecithin vesicles ( $2.5 \times 10^{-4}$  M phospholipid;  $R = 5$ ). Spectra were taken at  $20^\circ\text{C}$  in 7 mM Tris buffer pH 8.3.

#### Effect of the acyl chain length

In Fig. 3 *b* are presented the  $\Delta\pi$ - $\pi_i$  plots of the surfactin penetration into different phosphatidylcholine monolayers. The insertion of surfactin in the monolayer becomes more difficult when the lipid chain length increases. The exclusion pressure decreases from  $\approx 40 \text{ mN m}^{-1}$  for DMPC to  $\approx 30 \text{ mN m}^{-1}$  for DPPC and  $\approx 18 \text{ mN m}^{-1}$  for egg lecithin. The maximum surfactin penetration in DPPC monolayers induces a surface pressure increase of  $\approx 17 \text{ mN m}^{-1}$  and only  $10 \text{ mN m}^{-1}$  in egg lecithin monolayers. Adding cholesterol to the lecithin monolayer to have a lecithin/cholesterol 2:1 molar ratio does not change the exclusion pressure value.

#### Effect of charges

When surfactin is injected beneath a DMPA monolayer, the maximum  $\Delta\pi$  observed is only  $\approx 17 \text{ mN m}^{-1}$  instead of  $24 \text{ mN m}^{-1}$  in the case of DMPC (Fig. 3 *a*). Furthermore, the penetration of surfactin decreases very quickly with the lipid molecular packing as a result of the electrostatic repulsion between the peptide cycle of surfactin bearing two negative

charges and the polar head of DMPA. In fact, if we examine the adsorption kinetic of surfactin in a DMPA monolayer (Fig. 2 *a*) we observe first an important and instantaneous increase in surface pressure followed by a slow decrease (the same kind of trace is obtained with a PS monolayer, not shown) assuming that the surfactin and the phospholipid molecules get reorganized until a stable equilibrium state is reached.

The addition of  $\text{Ca}^{2+}$  ions (20 mM) in the subphase causes a significant increase of the surfactin insertion in PS monolayers with  $\Delta\pi$  reaching  $\approx 30 \text{ mN m}^{-1}$  instead of  $\approx 8 \text{ mN m}^{-1}$ . The  $\Delta\pi$ - $\pi_i$  plot presents a biphasic aspect: until  $\pi_i \approx 15 \text{ mN m}^{-1}$ , the slope is nearly the same with or without calcium ions, then  $\Delta\pi$  decreases more slowly as the molecular packing of PS increases (Fig. 3 *c*).

As shown in Fig. 4, the presence of  $\text{CaCl}_2$  (20 mM) in the subphase leads to an important change of the surfactin penetration process. The shape of the  $\Delta\pi$ - $C$  plot is very different from that observed when the subphase does not contain  $\text{Ca}^{2+}$  and the increase in surface pressure starts only when the surfactin bulk concentration reaches at least  $2 \times 10^{-7}$  M. We

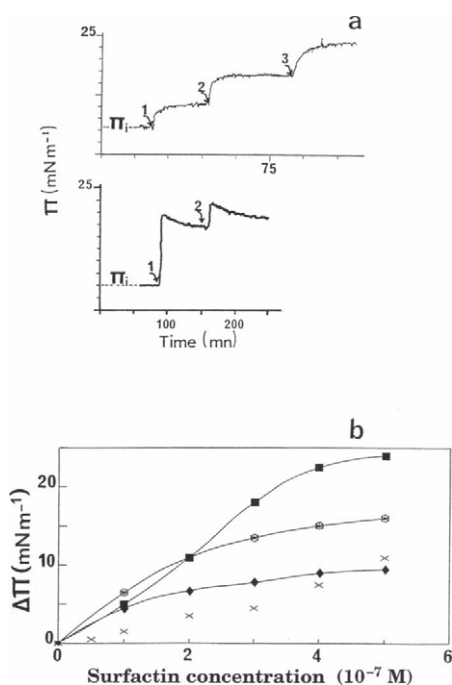


FIGURE 2 Changes of the surface pressure of a phospholipid monolayer upon the addition of surfactin in the subphase. (a) Traces showing the surface pressure increase of a phospholipid monolayer upon successive additions of surfactin in the subphase.  $\pi_i$  is the initial surface pressure of the phospholipid film. Aliquots of the surfactin solution are added beneath the film at the following final concentrations (arrows):  $10^{-7}$  M,  $2 \times 10^{-7}$  M, and  $3 \times 10^{-7}$  M. (top) DMPC monolayer; (bottom) DMPA monolayer. Subphase: 5 mM Tris buffer pH 8.5.  $T = 20^\circ\text{C}$ . (b) Surface pressure increase of a DMPC monolayer as a function of surfactin concentration in the subphase. Initial pressure of the DMPC monolayer: (■)  $5.5 \text{ mN m}^{-1}$ , (\*)  $14 \text{ mN m}^{-1}$ , (◆)  $25.5 \text{ mN m}^{-1}$ . (x) The surface pressure increase observed in the absence of lipid film, due to the adsorption of surfactin at the air/water interface.

have compared the surfactin penetration into various phospholipid monolayers in the expanded state ( $\pi_i \approx 5 \text{ mN m}^{-1}$ ). When phospholipids possess zwitterionic polar head and short acyl chains (DMPC and DMPE) the  $\Delta\pi$  value reached at  $C = 5 \times 10^{-7} \text{ M}$  is nearly the same with or without  $\text{CaCl}_2$  (Fig. 4 a). When phospholipids are zwitterionic but with longer acyl chains (DPPC and egg lecithin), we observe a jump in the  $\Delta\pi$  value at  $C = 5 \times 10^{-7} \text{ M}$ , and the greater the acyl chain length, the higher the  $\Delta\pi$  value (Fig. 4 b). When the polar head is acidic (DMPA and phosphatidylserine),  $\Delta\pi \approx 35 \text{ mN m}^{-1}$ , corresponding to a total monolayer surface pressure of about  $40 \text{ mN m}^{-1}$  (Fig. 4, c and d).

### Mixed surfactin/phospholipid spread monolayers

#### Mixed surfactin/DMPC monolayers

Isotherm curves of mixed surfactin/DMPC monolayers, spread at the air/water interface, have been plotted for different surfactin molar fractions  $x_s = n_s/(n_s + n_l)$  (where  $n$  is the number of molecules at the interface; the subscripts S and L refer to surfactin and lipid, respectively). Some of these curves are shown in Fig. 5 a.

Fig. 5 b plots the values of the mean molecular area of the mixed monolayers as a function of composition at a given pressure (data taken from the isotherms). These plots show rather small negative deviations from the additivity rule (Gaines, 1966) at moderate surface pressure values. However, we observe only a small variation of the transition pressure. So it is possible that the negative area deviations are the consequence of a smaller repulsion between surfactin molecules when mixed with DMPC. At higher surface pressures (from about  $20 \text{ mN m}^{-1}$ ), the mean molecular area,  $A$ , varies linearly with the composition,  $x_s$ , of the monolayer. The results are then more consistent with an ideal miscibility of DMPC and surfactin when spread together at the air/water interface than with the formation of a DMPC/surfactin complex.

#### Mixed surfactin/phosphatidylserine monolayers

Isotherm curves of mixed surfactin/phosphatidylserine monolayers spread on a subphase containing  $20 \text{ mM CaCl}_2$  are presented Fig. 6 a.

Fig. 6 b plots the values of the mean molecular area as a function of composition at  $10$ ,  $20$ , and  $30 \text{ mN m}^{-1}$ . The deviations to the additivity rule are in the range of the experimental errors, and we observe a small variation of the transition (or collapse) pressure with the composition of the monolayer (Fig. 6, inset). These results are interpreted as a lack of specific interactions between the two components.

## DISCUSSION

Surfactin is a very powerful biosurfactant, and its adsorption at the air/water interface, in the absence of any lipid monolayer, is far from being negligible (Maget-Dana and Ptak, 1992). At a bulk concentration of  $5 \times 10^{-7} \text{ M}$ , the surfactin adsorption induces, at a lipid-free solution surface, a surface pressure increase  $\Delta\pi_0 = 10 \text{ mN m}^{-1}$ . Then only  $\Delta\pi$  values  $>10 \text{ mN m}^{-1}$  signify an increased adsorption of surfactin at the interface due to the presence of the lipid monolayer. The difference  $\Delta\pi - \Delta\pi_0 = \Delta\pi_{\text{inter}}$ , called interaction pressure (Pethica, 1955; Anderson and Pethica, 1956), is related to interactions between the lipopeptide in solution and the spread monolayer compounds. From the data in Figs. 3 and 4, it can be seen that, in the absence of  $\text{CaCl}_2$ , only monolayers made of phospholipids possessing short and saturated acyl chains ( $C \leq 16$ ) are presumed to interact specifically with surfactin. These phospholipids have acyl chains of the same length and nature as the hydrophobic chain ( $C = 14$  or  $15$ ) of surfactin. It follows that the chain-chain interactions are optimum in the case of dimyristoyl phospholipids when the peptide cycle is at the level of the polar head groups (zwitterionic phospholipids). In egg lecithin monolayers, surfactin does not provoke an additional surface pressure increase (Fig. 3 b) because of steric hindrance due to unsaturation and ramification of the lipid acyl chains. In this case, the hydrophobic chain of surfactin cannot penetrate entirely into the lipid monolayer. When the lipid bears a negative

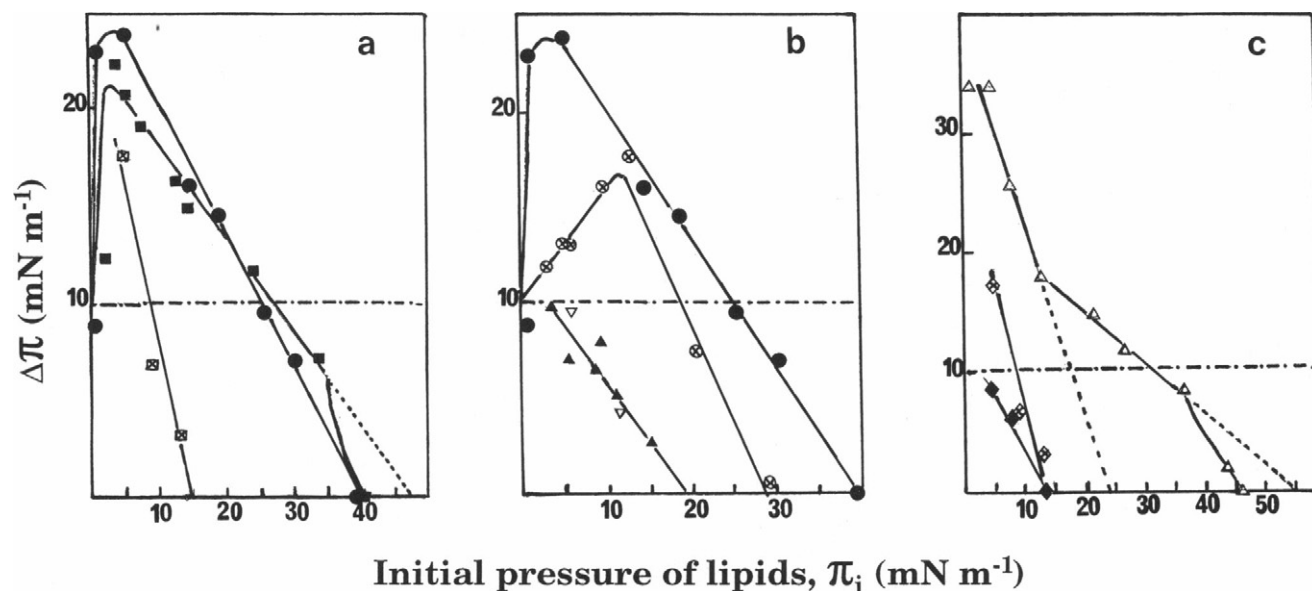


FIGURE 3 Insertion of surfactin in a phospholipid monolayer as a function of the initial pressure. Surface pressure increase ( $\Delta\pi$ ) upon addition of  $5 \times 10^{-7}$  M surfactin in the subphase. (a) Dimyristoyl phospholipids: (●) DMPC, (■) DMPE, (⊠) DMPA. (b) Lecithins: (●) DMPC, (⊗) DPPC, (▲) egg lecithin, (▽) egg lecithin/cholesterol 2/1. (c) (⊗) DMPA, (◆) beef brain PS, (△) beef brain PS in the presence of 20 mM  $\text{Ca}^{2+}$ . The line (---) represents the  $\Delta\pi$  value in the absence of lipid monolayer due to the adsorption of surfactin at the air/water interface. Subphase: 5 mM Tris buffer pH 8.5.  $T = 20^\circ\text{C}$ .

charge, the electrostatic repulsion prevents the peptide cycle from coming close to the phospholipid head groups.

This study outlines the role of divalent cations like  $\text{Ca}^{2+}$  in the membrane penetration process of surfactin. Ionized surfactin binds  $\text{Ca}^{2+}$  ions forming a surfactin-Ca 1:1 complex, where one  $\text{Ca}^{2+}$  ion makes a bridge between the two acidic residues (L-Glu<sup>1</sup> and L-Asp<sup>5</sup>) of the peptide cycle

(Maget-Dana et al., 1992; Thimon et al., 1992a, 1993). We have shown here that the calcium binding is accompanied by a conformational change of the peptide cycle. This new conformation is probably similar to that deduced from NMR techniques for the protonated surfactin with the two polar groups (Glu<sup>1</sup> and Asp<sup>5</sup>) facing each other (Bonmatin et al., 1994). The surfactin- $\text{Ca}^{2+}$  complex is a neutral compound that is not very soluble in water: its CMC is only  $2 \times 10^{-5}$  M instead of  $3 \times 10^{-4}$  M for ionized surfactin in the absence of ions (Thimon et al., 1992a). Therefore, the complex has a natural tendency to dissolve in the hydrophobic medium made up of phospholipid monolayers.

The changes in the CD spectra of surfactin upon addition of lipid vesicles can be attributed to conformational changes

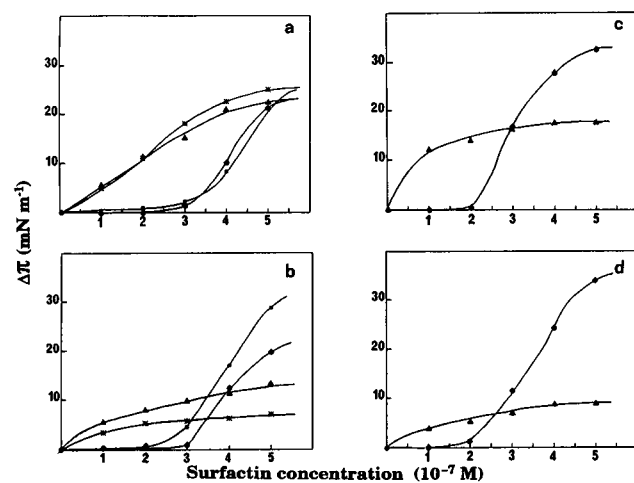


FIGURE 4 Effect of Calcium ions on the insertion of surfactin in phospholipid monolayers. (a) zwitterionic dimyristoyl phospholipids: DMPC, in the absence (\*) and in the presence (■) of  $\text{Ca}^{2+}$  ions. DMPE, in the absence (△) and in the presence (●) of  $\text{Ca}^{2+}$  ions. (b) Lecithins: DPPC, in the absence (△) and in the presence (●) of  $\text{Ca}^{2+}$  ions. Egg lecithin in the absence (\*) and in the presence (■) of  $\text{Ca}^{2+}$  ions. (c) DMPA, in the absence (△) and in the presence (●) of  $\text{Ca}^{2+}$  ions. (d) Beef brain PS, in the absence (△) and in the presence (●) of  $\text{Ca}^{2+}$  ions. The initial pressure of the lipid monolayers is  $\pi_i = 5 \pm 0.5 \text{ mN m}^{-1}$ . Subphase: 5 mM Tris buffer pH 8.5.  $T = 20^\circ\text{C}$ . Surfactin is added in the subphase beneath the phospholipid monolayer.

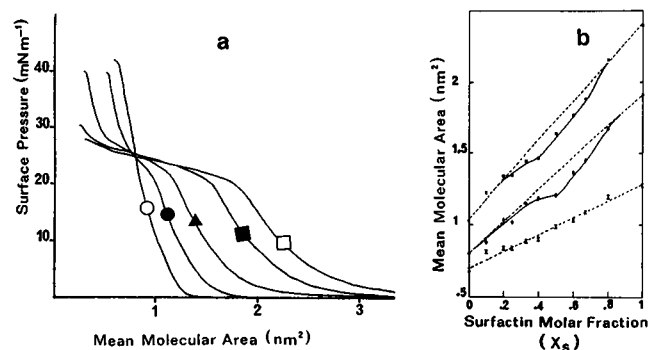


FIGURE 5 Mixed surfactin/DMPC monolayers. (a) Compression isotherm curves. The surfactin molar fraction  $X_s$  is: (□) 1 (pure surfactin); (■) 0.8; (▲) 0.5; (●) 0.25; (○) 0 (pure DMPC). (b) Mean molecular areas as a function of composition at various surface pressures: (■)  $5 \text{ mN m}^{-1}$ ; (◆)  $15 \text{ mN m}^{-1}$ ; (X)  $23 \text{ mN m}^{-1}$ . The line (---) represents the additivity rule values (arithmetic mean of surfactin and DMPC molecular areas). Subphase: 5 mM Tris buffer pH 8.5.  $T = 20^\circ\text{C}$ .

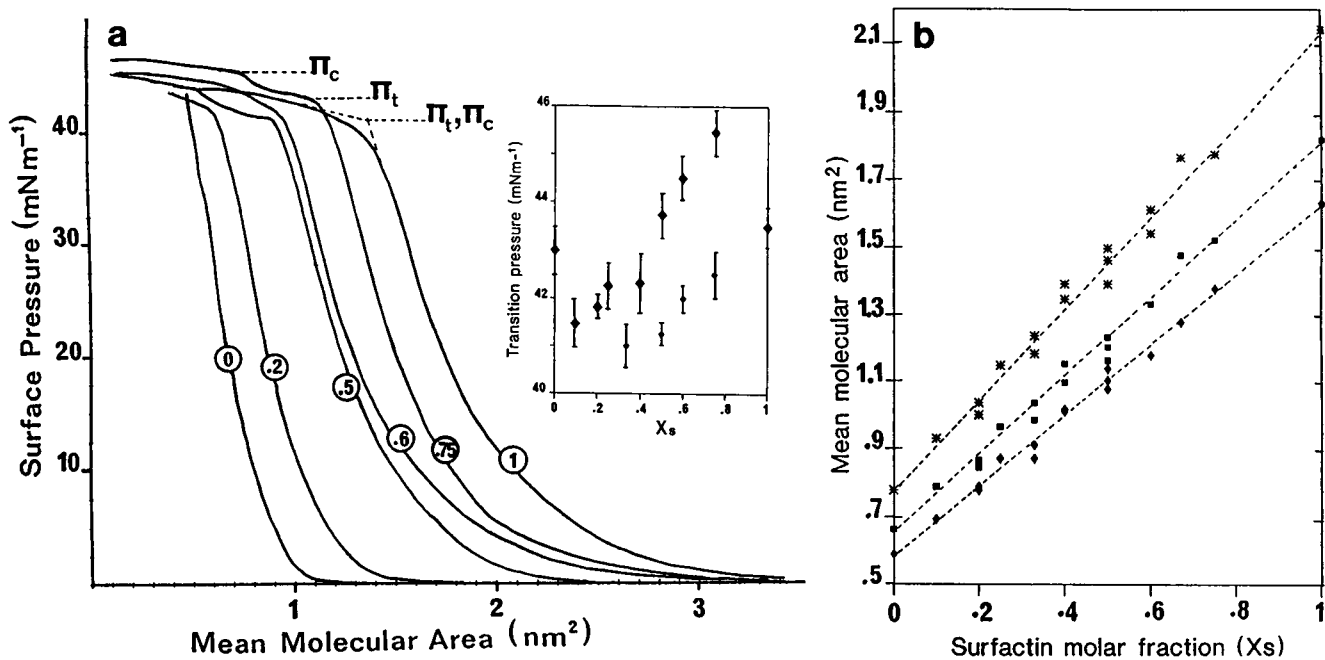


FIGURE 6 Mixed surfactin/PS monolayers in the presence of  $\text{Ca}^{2+}$  ions (20 mM) in the subphase. (a) Compression isotherm curves. The surfactin molar fraction  $X_s$  is indicated on the curves: (0) pure PS; (1) pure surfactin.  $\pi_t$ : transition pressure;  $\pi_c$ : collapse pressure. (inset) Variation of the transition (or collapse) pressure as a function of composition; (●)  $\pi_t$ ; (◆)  $\pi_c$ . (b) Mean molecular areas as a function of composition at various surface pressures: (\*) 10  $\text{mN m}^{-1}$ ; (■) 20  $\text{mN m}^{-1}$ ; (◆) 30  $\text{mN m}^{-1}$ .

of the peptide cycle when it interacts with lipid membranes. The magnitude of the CD change is greater in the presence of calcium ions, assuming that the surfactin-Ca 1:1 complex is more deeply incorporated into the lipid bilayer. Therefore, in addition to reduce electrostatic repulsion, the presence of  $\text{Ca}^{2+}$  ions contributes to promote hydrophobic interactions between surfactin and phospholipid acyl chains, and the longer the chain the higher the penetration.

## CONCLUSION

The main point of this study is the significant effect of calcium on both the conformation of surfactin and its insertion into lipid membranes. These results agree with the fact that the biological properties of surfactin, especially erythrocyte hemolysis, are also greatly enhanced by the presence of calcium (Thimon et al., 1992a). One can suppose that surfactin (as the surfactin-Ca complex) acts first by penetrating readily into the cell membrane, where it is completely miscible with the lipid components. Surfactin interacts with lipids, mainly by means of hydrophobic interactions. However, we failed to demonstrate the formation of complexes with phospholipids. In that, surfactin differs from iturin compounds, another class of antibiotic lipopeptides possessing a very similar structure (Maget-Dana et al., 1989; Maget-Dana and Ptak, 1990). Our results are in agreement with those of Sheppard et al. (1991), who found that the ion-conducting pores induced by surfactin are not due to the formation of lipid/surfactin structures in the lipid bilayer but, rather, to the presence of surfactin dimers. The complexation with  $\text{Ca}^{2+}$  could

promote the formation of these dimers. In addition to conducting pores, the conductance jumps observed by Sheppard et al. (1991) could be related to the ion-carrier properties of surfactin (Thimon et al., 1993).

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