Computer Simulation of Surfactin Conformation at a Hydrophobic/Hydrophilic Interface

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Using a molecular modeling method, different conformations of surfactin at a hydrophobic/hydrophilic interface are established. Two conformations of the peptide ring (S1 and S2) provided by NMR experiments built with three different aliphatic chains in folded or extended configurations were studied. For the structures including the S2 peptide ring conformation, the theoretical interfacial molecular area corresponds to the experimental limiting area A_0 value obtained with a Langmuir film balance. The peptide ring is positioned in the plane of the interface with the two acidic chains close to each other and protruding in the aqueous phase, and the β-hydroxy fatty acid chain, folded to interact mainly with the Leu2 side chain and also with the Val4 side chain. This design has the largest calculated molecular area and would correspond to the most stable amphipathic structure representing the surfactin experimental behavior in weak compression.

Introduction

Surfactins are lipopeptides produced by *Bacillus subtilis*. Their structure is composed of a lactone ring system formed by a heptapeptide and a C_{13} to C_{15} β-hydroxy fatty acid (Figure 1).[1]

The amphiphilic character of the molecules, with a hydrophobic part consisting of a long fatty acid chain and some lipophilic amino acids (Leu2, Leu3, Val4, Leu6, Leu7) and a hydrophilic part with the backbone of the cycle and two anionic residues (Glu1 and Asp5), explains their very powerful surfactant properties. Surfactins have a strong tendency to adsorb at hydrophilic/hydrophobic interfaces and consequently can reduce the surface tension of aqueous solutions. In fact, they can decrease the surface tension of distilled water from 72 to 27 mN/m.[2] They have also excellent foaming properties,[3] a macroscopic consequence of their surface activity.

In addition to their biosurfactant character, surfactins exhibit various interesting biological effects such as antibacterial,[1] antimycoplasma,[4] and antiviral[5] activities, in vitro haemolytic activity,[1,6,7] inhibition of blood clotting,[2] hypocholesterolemic properties,[8] and cAMP phosphodiesterase inhibitory properties.[9] Some of these effects can be explained by the ability of surfactins to interact with phospholipids[10] and to induce the formation of selective cationic pores in phospholipid bilayers.[11]

Surface-active and biological properties mainly occur at interfaces and are governed by the conformational state

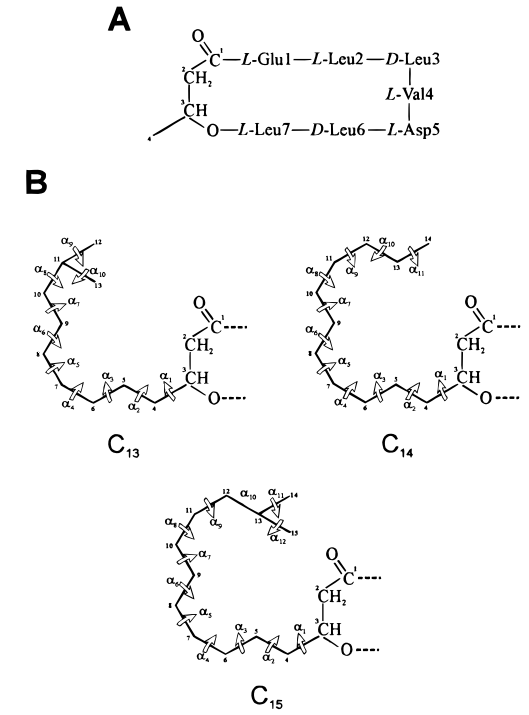

Figure 1. (A) Primary structure of surfactin. The aliphatic chain contains only the C_3 and C_4 carbon atoms as considered by Bonmatin et al.[14] (B) Primary structures of the three different aliphatic chains (C_{13}, C_{14}, and C_{15}) studied in this work showing the numbering of the carbon atoms of the β-hydroxy fatty acid side chain and the torsional angles (α_i).

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of molecules at hydrophobic/hydrophilic interfaces.[12,13] Consequently, the surfactin tridimensional structure determination at a hydrophobic/hydrophilic interface is essential.

Several conformational studies of surfactins in solution or in the dry state have been presented,[14,15] and a surfactin conformation at the air/water interface has been suggested.[14,16] However, the study of the whole surfactin tridimensional structure at interface on the basis of an energetic criterion has not yet been carried out.

By a two-dimensional ^{1}H-NMR study in dimethyl sulfoxide (DMSO) combined with molecular modeling, Bonmatin et al.[14] proposed two possible conformations for surfactin (S1 and S2). Both structures are characterized by a "horse saddle" topology for the ring atoms on which are attached the two polar glutamic and aspartic side chains pointed at the opposite direction of the methyl group which truncates the aliphatic chain. The major difference between the two backbones is related to the hydrogen bond network. In S1, the structure exhibits a single hydrogen bond [NH(5)−CO(2)] characterizing a β-turn for the sequence Leu2−Leu3−Val4−Asp5 whereas a network of three hydrogen bonds [NH(7)−CO(5), NH(4)−CO(2), NH(6)−C^{1}O] is observed in S2.

Ferré et al.[15] performed conformational studies of surfactin by Fourier transform infrared spectroscopy in the dry state, ^2H_2O, dimethyl sulfoxide, and trifluoroethanol. Their results in DMSO point out the presence of a β-turn in the lipopeptide.

A molecular model of surfactin at an air/water interface based on primary structure from mass spectroscopy and amphiphilic properties was assumed by Ishigami et al.[16] Following their opinion, surfactin molecules form dimers by hydrophobic interactions between aliphatic chains. Without compression, molecules orientate themselves with the peptide ring and aliphatic chains lying flat on the subphase surface. When compressed, they take up a close packing configuration with the aliphatic chains normal to the peptide ring lying on the subphase surface. This is in contradiction with the assumption of Bonmatin et al.,[14] which presents both the peptide ring and the aliphatic chains standing vertically on the water surface when molecules are under compression conditions.

In the present study, we propose a conformational model of the whole surfactin structures at a hydrophobic/hydrophilic interface to gain a better understanding of the surface-active and biological properties of surfactin. A theoretical method was applied on NMR data to build up the most stable conformation at a hydrophobic/hydrophilic interface. The molecular areas calculated from the models were compared to those obtained from Langmuir film balance experiments.

Both methods were applied on three surfactin homologues to display the potential influence of the aliphatic chain hydrophobicity on the molecule conformation at a hydrophobic/hydrophilic interface.

Materials and Methods

Computation of the Structures. Atomic coordinates of the surfactin ring S1 and S2 structures were provided by Bonmatin et al.[14] Carbon atoms of the β-hydroxy fatty acid chain were added to the surfactin structures using the Hyperchem 5.0 software (Autodesk, Sausalito, CA). The literature contains several mistakes about the carbon atoms number of the β-hydroxy fatty acid chain of the surfactin. The number of carbon atoms of a β-hydroxy fatty acid chain is accurately counted from the carbon atom of the acidic moiety. Thus, the first carbon atom in the β-hydroxy fatty acid chain of the surfactin is the one bound to the nitrogen atom of the glutamic residue. Consequently, the numbers of carbon atoms located out of the ring in the C_{13}, C_{14}, and C_{15} surfactins are 10, 11, and 12, respectively.

The method used for the theoretical analysis of the surfactin conformations is based on a semiempirical method.[17] The total conformational energy that represents the sum of the van der Waals interactions, the torsional potential, and the electrostatic interactions is calculated for a large number of conformations in a systematic analysis bearing on all torsional angles (α) (Figure 1). Those angles were affected by systematic 60° changes using two successive analyses. The first one was performed on angles α_1−α_6 (in the C_{13}−C_{15} aliphatic chains) generating 46 656 (6^6) conformations. The second analysis was performed on angles from α_5 to α_8 (C_{13} chain) and from α_5 to α_9 (C_{14} and C_{15} chains) generating 1296 (6^4) and 7776 (6^5) conformations, respectively. In total, 47 952 conformations were generated for the C_{13} structures and 54 432 for the C_{14} and C_{15} structures. Figure 2A/C summarizes the most probable configurations for each structure (selection based on a Boltzmann statistical weight of all configurations) with their probability of existence in a structure tree[18] obtained after the two systematic analyses. Conformations with probabilities of existence <5% were discarded. Selected conformations for each structure tree were then submitted to a simplex minimization procedure.[19] This calculation was carried out in a medium of intermediate dielectric constant of a hydrophobic/hydrophilic interface. The most probable conformation based on a Boltzmann statistical weight was selected, and its orientation at the hydrophobic/hydrophilic interface was defined by calculations of the hydrophobic and hydrophilic centers as described elsewhere.[17] The conformations of surfactin molecules assembled in monolayer were established using the hypermatrix procedure from Tammo (Theoretical Analysis of Molecular Membrane Organisation) software as detailed elsewhere.[20] The position of the central surfactin molecule was fixed and a layer of similar molecules was added one by one around it in order to maintain a minimal energy. Interfacial molecular area values of assemblages were calculated using Tammo software.[21] All molecule visualizations were performed on PC 586, using WinMGM software[22] from Ab Initio Technology (Obernai, France).

Compression Isotherm Curves. The surfactins production and extraction were carried out as detailed in Razafindralambo *et al*.[23] Primary structure and purity of the surfactin homologous series (>95%) were ascertained by analytical RP-HPLC (Chromspher 5 μm C18 column, 1 × 25 cm, Chrompack, Middelburg, The Netherlands), amino acid analysis, and electrospray mass spectrometry measurements using a Finnigan MAT 900 ST. Three surfactin homologues were obtained, two comprising a branched β-hydroxy fatty acid chain (isopropyl group at the chain end) with 13 (SuC_{13}; MW 1008) or 15 (SuC_{15}; MW 1036) carbon atoms and one enclosing a linear β-hydroxy fatty acid chain with 14 carbon atoms (SuC_{14}; MW 1022) (Figure 1).

Compression isotherm curves of lipopeptide films were established using a Langmuir film balance (LFW2 3″5-Lauda, Königshofen, Germany) containing milliQ water (Millipore Co., Milford, MA) adjusted at pH 2.0 with HCl as a subphase.

Surfactin was dissolved in *n*-hexane/chloroform (2/1, v/v). Solutions of 30 μL were spread on the subphase with a

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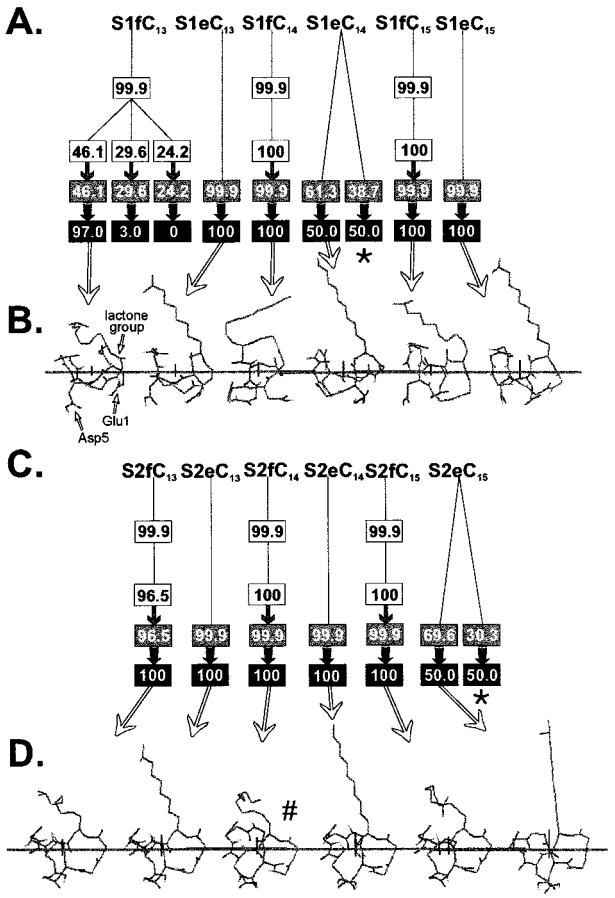

Figure 2. (A/C) Structure tree of the S1 and S2 surfactins, respectively. The structure tree was obtained after two successive conformational analyses for the folded β-hydroxy fatty acid chain ($S1fC_{13}$, $S1fC_{14}$, $S1fC_{15}$, $S2fC_{13}$, $S2fC_{14}$, and $S2fC_{15}$) and one conformational analysis for the extended β-hydroxy fatty acid chain ($S1eC_{13}$, $S1eC_{14}$, $S1eC_{15}$, $S2eC_{13}$, $S2eC_{14}$, and $S2eC_{15}$). Probabilities of existence (Boltzmann) for each systematic analysis are reported in white boxes. The sum of the probabilities for each branch are reported in gray boxes. Probabilities of existence after the simplex procedure are reported in black boxes. (B/D) Orientation at the hydrophobic/hydrophilic interface (horizontal line) of the most probable conformation of S1 and S2 surfactins, respectively, after a simplex minimization procedure and a selection based on a Boltzmann statistical weight. The hydrophilic medium is located under the interface. The sign asterisk indicates that the structure was rejected because the assemblage was not proper: molecules tend to overlap and some of them are completely in the hydrophobic medium which does not correspond to the experimental fact. The number sign shows the structure $S2C_{14}$ assembled in Figure 3.

microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland). After a period of 15 min to ensure the complete evaporation of the solvent, the film was compressed at a 61.8 $cm^2 \cdot min^{-1}$ rate. All measurements were carried out at 20 ± 0.5 °C. Each measurement was replicated three times.

Results and Discussion

Theoretical Results. The molecular structure and the numbering of the torsional angles ($\alpha_1-\alpha_{12}$) are represented in Figure 1. Molecular modeling method was performed on the S1 and S2 structures obtained by NMR experiments.[14] In both conformations, the acidic groups are in a protonated form. They are closer in S2 (3.4 Å) than in S1 (6.1 Å). Only the C_3 and C_4 atoms of the aliphatic chain exist in those structures (Figure 1A). Nine to eleven carbon atoms were added according to the length of the β-hydroxy fatty acid chain (from C_{13} to C_{15}) (Figure 1B). Those containing 13 or 15 carbon atoms are isobranched, and the one with 14 carbon atoms is linear in order to be compatible with molecules used in the experimental part. Six different structures of surfactin were built according to the peptide ring conformation (S1 or S2) and the number of carbon atoms in the β-hydroxy fatty acid chain (13−15 carbon atoms). Only conformations of the β-hydroxy fatty acid chain were calculated; structures of the peptide ring were not changed.

In the energy-refined structures (called $S1fC_{13}$, $S1fC_{14}$, $S1fC_{15}$, $S2fC_{13}$, $S2fC_{14}$, and $S2fC_{15}$; Figure 2B/D), the β-hydroxy fatty acid chain is folded to interact at best with hydrophobic side chains of the peptide ring and thus to minimize apolar surfaces of the molecule accessible to the solvent. The β-hydroxy fatty acid chain of S2f structures interact mainly with the Leu2 side chain and also with the Val4 side chain. In the S1f structures, the folding of the β-hydroxy fatty acid chain differs according to the number of carbon atoms in the chain.

Another systematic analysis was carried out only on the α_1 angle of each surfactin structure. This procedure enables one to obtain extended conformations of the β-hydroxy fatty acid chain. The structures were called $S1eC_{13}$, $S1eC_{14}$, $S1eC_{15}$, $S2eC_{13}$, $S2eC_{14}$, and $S2eC_{15}$ (Figure 2B/D).

Hydrophobic and hydrophilic baricenters were mapped for each conformation, and a hydropathy interface was drawn. This plane was used to predict the orientation of the surfactin molecules at a hydrophobic/hydrophilic interface. Twelve conformations were analyzed: the S1f and S2f structures (folded β-hydroxy fatty acid chains) and the S1e and S2e structures (extended β-hydroxy fatty acid chains), each with three different aliphatic chains (C_{13}, C_{14}, and C_{15}). Their orientation is shown in Figure 2B/D. In general, the β-hydroxy fatty acid chain and the lactone group are located in the hydrophobic medium. Whatever the size of the β-hydroxy fatty acid chains, the peptide ring of all the S2 structures lies flat with respect to the hydrophilic/hydrophobic interface with both acidic residues in the hydrophilic medium. In the S1 structures, peptide ring orientations at the interface are different. The ring plane of the $S1fC_{14}$ structure is located perpendicular to the interface. Consequently, the glutamic side chain (Glu1) lies in the hydrophobic medium while the aspartic residue (Asp5) is located on the hydrophilic side. The peptide ring of the $S1fC_{15}$ structure is slighly tilted with respect to the interface with several apolar side chain and the two acidic chains pointing toward the hydrophilic medium. About the $S1fC_{13}$ and S1e structures, the molecule lies flat with respect to the interface with both acidic side chains in the hydrophilic medium. The different positions of the surfactin molecule according to the hydrophobic/hydrophilic interface are related with the variable conformations of both the aliphatic chain and the two acidic side chains. Unlike the S1f and S1e structures, the conformation of the aliphatic chain in the S2f and S2e structures does not influence the orientation of the peptide ring at the interface. The closeness of the two acidic side chains in S2f and S2e structures forms a highly distinct polar domain that counterbalances the hydrophobic β-hydroxy fatty acid side chain and displays the more amphipathic character of the molecule in comparison with the S1f and the S1e structures.

To approach the monolayer features obtained in our experimental study, we performed a multimolecular assemblage of surfactin molecules according to the plane of the hydrophilic/hydrophobic interface. Four to seven molecules were matched around a central one. An example of multimolecular assemblage of seven molecules of $S2fC_{14}$

Table 1. Interfacial Molecular Areas (in Å²) of C_{13}, C_{14}, and C_{15} Surfactins Obtained by Theoretical and Experimental Methods[a]

			C_{13}		C_{14}		C_{15}	
		ring alone	folded	extended	folded	extended	folded	extended
theor	A_{S1}	131	143	126	144	137	138	126
values	A_{S2}	129	157	150	153	149	156	147
exptl	A_0		154.2 ± 1.9		161.7 ± 1.2		158.0 ± 2.0	
values	A_t		103.7 ± 2.5		119.5 ± 0.5		112.0 ± 0.5	

[a] The theoretical values correspond to molecular areas of surfactins assembled in a monolayer including either the S1 or the S2 peptide ring conformation. The experimental areas A_0 and A_t are determined from curves shown in Figure 4.

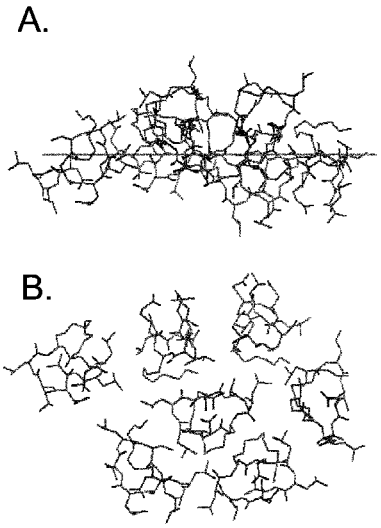

Figure 3. Side view (A) and top view (B) of the multimolecular assemblage of surfactin (conformation S2fC_{14}). The central surfactin molecule position is fixed, and layers of similar molecules are added one by one around it in order to maintain a minimal energy. Seven molecules were assembled. The hydrophobic/hydrophilic interface is shown only in the side view (horizontal line) and has been removed in the top view for clarity. The hydrophilic medium is located under the interface.

is shown in Figure 3. Unlike the Ishigami et al.[16] hypothesis, this assemblage does not display dimer formation between two surfactin molecules.

The molecular interfacial areas were calculated for every assemblage (Table 1). S2 interfacial areas are greater than S1 interfacial areas. In S1 structures, interfacial area values of folded conformations are superior than the one of extended conformations. However in S2 structures, the folded and extended conformations have similar interfacial areas because the orientation of the peptide ring is not influenced by the folded or extended configuration of the β-hydroxy fatty acid chain. Moreover, in S2 structures, area values are not influenced by the increase of the aliphatic chain hydrophobicity (13−15 carbon atoms). It could be expected that the interfacial areas of the peptide ring without aliphatic chain would be similar to the extended conformation one. This hypothesis is true for S1 structures but not for S2 structures. We can then assume that, for S2 structures, the addition of an aliphatic chain to the peptide ring plays a primordial role in the molecule position and orientation at the interface.

The theoretical area of the surfactin molecule at interface determined by Ishigami et al.[16] is higher than what we observed for S1 and S2 structures. Moreover, our theoretical interfacial area of the peptide S1 and S2 rings without aliphatic chain is smaller than those reported by Bonmatin et al. (150 Å² for S1 and 220 Å² for S2).[14] The position and the orientation of surfactin molecules at the interface may explain these discrepancies.

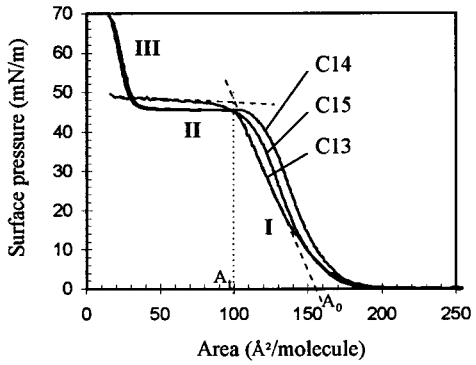

Figure 4. Compression isotherm curves of C_{13}, C_{14}, and C_{15} surfactins established with a Langmuir film balance. The subphase is milliQ water at pH 2.0, and the temperature is 20 °C.

Bonmatin et al.[14] and Ishigami et al.[16] have positioned the molecule at the interface on the basis of experimental assumptions without taking into consideration the energetic criterion. Their theoretical area values fit with their experimental values although the cations, which are present and exert an influence on the results in experiments,[24] are not taken into account in their model. Moreover, Bonmatin et al.[14] have truncated the aliphatic chain by a methyl group in their model. In our case, no cation interferes either in experimental or theoretical assays, and the position and the orientation at the interface of the surfactin molecule comprising the aliphatic chain is carried out by energetic minimization.

Experimental Results. In the experimental part, the pressure−area plots of SuC_{13}, SuC_{14}, and SuC_{15} isotherm monolayers are recorded with a Langmuir film balance. Curves are shown in Figure 4. Under the conditions used, surfactins are protonated.

The shape of curves is analogous for the three surfactin homologues and similar to those obtained by Maget-Dana and Ptak,[25] who worked on a mixture of surfactin homologues, and Ishigami et al.,[16] who worked on C_{15} surfactin.

Curves present three parts. Part I of the isotherm corresponds to an expanded monolayer. This part is almost linear and approaches the $\pi = 0$ axis at a fairly steep angle. The intersection of the tangent to this part with the area axis defines the limiting interfacial molecular area A_0. Further compression results in a sharp break and the formation of a fairly horizontal plateau (part II). The intersection of the tangents to part I and part II corresponds to the transition point (A_t, π_t). At the end of the plateau, there is a vertical part (part III) in the isotherm compression corresponding to a condensed state of the monolayer.

At A_0, molecules are adequately closed to exert an action on surface pressure. The A_0 value gives thus an indication

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of the cross-sectional area of surfactin molecules when they begin to take a regular orientation without being closely packed.

The transition point would represent a conformational change of molecules which permits a closer packing of molecules. The significance of the transition state is not well understood. According to Langmuir,[26] the beginning of the transition occurs because some of the molecules in the expanded film become organized into clusters or two-dimensional micelles. As the film is further compressed, the number of micelles is assumed to increase until the entire film is condensed.

In the condensed state, molecules are arranged in their closest possible packing. This reflects the presence of strong interactions between molecules.

Characteristic areas (A_0 and A_t) were determined from curves. They are presented in Table 1. The experimental values are similar, from 154.2 Å^2 (SuC_{13}) to 161.7 Å^2 (SuC_{14}) for A_0 and from 103.7 Å^2 (SuC_{13}) to 119.5 Å^2 (SuC_{14}) for A_t. The $\pi-$A behavior of surfactin monolayer seems thus not governed by the hydrophobic character of the β-hydroxy fatty acid chain under our experimental conditions.

The area values reported for mixture of surfactin homologues by Maget-Dana and Ptak[25] are 215 Å^2 for A_0 and 132 Å^2 for A_t at 10 °C with a 10 mM citrate buffer at pH 3.03. Ishigami et al.[16] report an A_0 value of 184 Å^2 and an A_t value of 89 Å^2 for C_{15} surfactin at 20 °C with a 10 mM citrate buffer at pH 4.2. The subphase used by these authors contains cations which can exert an influence on film expansion[24] while ours does not.

Confrontation of Theoretical and Experimental Results. The calculated interfacial areas of folded S2f conformations are similar to the limiting areas A_0 obtained from experimentation. They fit better than those of folded S1f conformations. Moreover, the distribution of the hydrophilic and hydrophobic parts is more distinct in the S2 conformation than in S1. The S2f conformation in the folded design would be thus the most stable and most likely structure at the interface for surfactin under weak compression. In this design, increasing hydrophobicity of the β-hydroxy fatty acid chain does not influence the interfacial molecular area as also observed in experimental results. Furthermore, the S2f structure in this model presents a molecular area higher than the S1f one and therefore better justifies the ability of surfactin to lower the surface tension of water from 70 to 27 mN/m.[2] The conformation of the S2f molecules, with the peptide ring lying flat at the interface and the acidic side chains protruding in the hydrophilic phase, is also adapted to bind cations which can be found in the hydrophilic medium. Due to the poor correlation between the experimental and theoretical area value for the S1f structure, we suggest that these structures cannot correspond to stable conformations at the interface but may rather be related to states of surfactin in solution.

The calculated area values of the extended structures (S1e and S2e) could be expected to correspond to the A_t experimental values, but they are superior. This is probably due to the fact that the theoretical assemblage is formed by a restrained number of molecules. With a higher number of molecules in the assemblage, the molecules could be more closely packed as in the compression condition at the transition point in the experimental Langmuir film balance assays.

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Conclusions

By means of a theoretical method, 12 different conformations of surfactin were modelized at a hydrophobic/hydrophilic interface: two peptide rings (S1 or S2) and three β-hydroxy fatty acid chains in two configurations, folded or extended, were tested. Afterward, assemblages of these molecules were carried out. The interfacial areas of molecules in the assemblage were calculated and compared to these obtained in experimental assays with a Langmuir film balance. The experimental assays were performed on three forms of the surfactins: two of them contain a branched β-hydroxy fatty acid chain enclosing 13 or 15 carbon atoms, and the last contains a linear β-hydroxy fatty acid chain comprising 14 carbon atoms.

The surfactins with the peptide ring S2 and the β-hydroxy fatty acid chain in the folded configuration present an interfacial area similar to those obtained in the experimental assay.

Thus, the folded S2f conformations assembled in a monolayer would correspond to the most stable amphipathic structure representing the surfactin experimental behavior in weak compression. In this model, the peptide ring is positioned in the plane of the interface with the two acidic chains close to each other and protruding in the aqueous phase and the β-hydroxy fatty acid chain folded to interact mainly with the Leu2 side chain and also with the Val4 side chain.

On the basis of the theoretical results, we assumed that the addition of an aliphatic chain influences the position of the peptide ring at the interface but that the increase of the β-hydroxy fatty acid chain hydrophobicity has not a significant effect on the interfacial conformation as also observed in the experimental part.

Our study demonstrates that the computational approach is a valuable tool to obtain a rapid and clear picture of the orientation of surfactin in a monolayer which can explain the surface-active behavior of these molecules.

These results will also be useful for further investigations dealing with the surfactin conformation in phospholipid monolayer and bilayers.

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