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Molecular organization of surfactin–phospholipid monolayers: Effect of phospholipid chain length and polar head

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Abstract

Mixed monolayers of the surface-active lipopeptide surfactin-C_{15} and various lipids differing by their chain length (DMPC, DPPC, DSPC) and polar headgroup (DPPC, DPPE, DPPS) were investigated by atomic force microscopy (AFM) in combination with molecular modeling (Hypermatrix procedure) and surface pressure-area isotherms. In the presence of surfactin, AFM topographic images showed phase separation for each surfactin–phospholipid system except for surfactin–DMPC, which was in good agreement with compression isotherms. On the basis of domain shape and line tension theory, we conclude that the miscibility between surfactin and phospholipids is higher for shorter chain lengths (DMPC > DPPC > DSPC) and that the polar headgroup of phospholipids influences the miscibility of surfactin in the order DPPC > DPPE > DPPS. Molecular modeling data show that mixing surfactin and DPPC has a destabilizing effect on DPPC monolayer while it has a stabilizing effect towards DPPE and DPPS molecular interactions. Our results provide valuable information on the activity mechanism of surfactin and may be useful for the design of surfactin delivery systems.

Keywords: Langmuir film; Atomic force microscopy; Molecular modeling; Miscibility; Stabilizing effect; Nanoscale resolution

1. Introduction

Surfactin (Fig. 1A) is a lipopeptide produced by various *Bacillus subtilis* strains. It is composed of a heptapeptide cycle (*L*-Glu–*L*-Leu–*D*-Leu–*L*-Val–*L*-Asp–*D*-Leu–*L*-Leu) closed by C_{13}–C_{15} hydroxy fatty acid forming a lactone ring system. Surfactin exhibits strong surface activity and important biological properties [1–7]. The interactions of surfactin with biological membranes are known to determine its biological activity and involve insertion into lipid bilayers, modification of membrane permeability by channels formation or by carrying mono- and divalent cations, and membrane solubilization by a detergent-like mechanism [8,9].

As the activity of surfactin occurs within the bilayer, it is interesting to investigate the behaviour of surfactin inside the lipid matrix, after its penetration process. Previous interfacial properties measurements and molecular modeling have provided valuable insight into the miscibility and molecular orientation of mixed surfactin-dipalmitoylphosphatidylcholine (DPPC) monolayers [10,11]. As known, lipid fraction of biological membranes is mainly composed of phospholipids varying by their chain length and their ionic character, which might have an influence on surfactin activity.

Two former studies have already been devoted to the lipid specificity of the interaction of surfactin with biological membrane models [9,12]. Although they give valuable information, contradictions between the two papers exist, and

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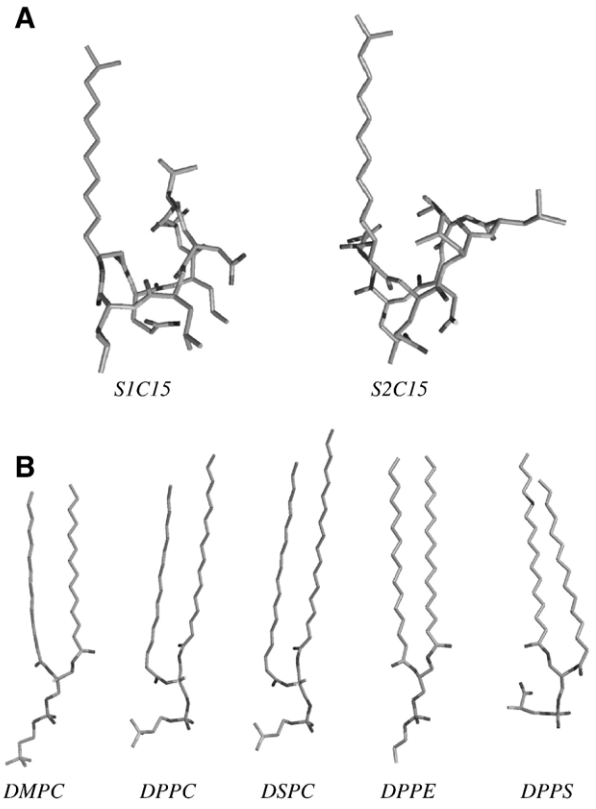

Fig. 1. Molecular models used in the multimolecular assemblies. (A) surfactin models; (B) phospholipid models.

particularly concerning the effect of surfactin on the interfacial organization of phospholipids. In the case of mixed monolayers of surfactin and dimyristoylphosphatidylcholine (DMPC), Maget-Dana and Ptak conclude that the two interfacial components are ideally miscible [9]. In other words, surfactin and DMPC do not specifically interact with each other and do not form a molecular complex. In contrast to their work, Grau et al. report the presence of specific molecular interactions between the DMPC acyl chains and the lipopeptide by performing differential scanning calorimetry on mixed surfactin–DMPC vesicles prepared in physiological conditions [12]. Such interactions are responsible of lateral phase separation of surfactin-rich domains within the bilayer. According to them, the domains are formed by clusters of surfactin and DMPC with a defined stoichiometry. Strong interaction between surfactin molecules is assumed to be at the origin of the phenomena. From their data, they did not see any pronounced difference between surfactin effects on DPPC and distearoylphosphatidylcholine (DSPC).

These two studies required further experimental data in order to reveal the presence or the absence of molecular interactions between the different interfacial components and consequently, to clarify their mixing behaviour. In this context, we have recently probed the interfacial behaviour of phospholipid monolayers following penetration of surfactin by using atomic force microscopy (AFM) [13]. Indeed, the ability of AFM to image surfaces at a nanometer lateral scale resolution and Angstrom vertical resolution makes it the technique of choice to distinguish domains in phase-separated films [14,15]. The presence or absence of domains and their morphology provide valuable information about molecular packing and organization of the components at the interface as well as about their miscibility on basis of the line tension theory. In this previous study, we showed that under physiological conditions the penetration power of surfactin and the nanoscale organization of the interfacial components at high surface pressure are more sensitive to the lateral arrangement of the phospholipids than to their chain length. In that work, AFM imaging was performed after surfactin penetration without being able to control the amount of lipopeptide inserted in the phospholipid monolayers and the transfer surface pressure of the mixed monolayers. As these two parameters play an important role in the mixing behaviour of the interfacial components, we propose, in the present study, to probe by AFM the interfacial organization of premixed surfactin–phospholipid monolayers spread with a defined molar ratio and compressed at a defined surface pressure.

Besides AFM, the Langmuir trough technique is used in order to have additional information on the interfacial properties of the film (miscibility, complex formation, thermodynamic stability) [10,16–18] in our experimental conditions. A procedure of molecular modeling (Hypermatrix procedure) is also applied to visualize at an atomic level the effect of surfactin on the lipid organization and to calculate the interaction energy in order to quantify the relative affinities between molecules and the stabilization effect of surfactin [11,19,20].

In addition to the effect of the phospholipid chain length, we also analyzed the influence of the phospholipid polar head on the interfacial behaviour of surfactin. Grau et al. have shown by performing DSC and X-ray diffraction experiments that addition of surfactin to dielaidoylphosphatidylethanolamine (DEPE) tends to destabilize the H_{II} structure [12]. Although their results do not show it explicitly, they conclude that surfactin is able to stabilize the DEPE bilayer. In our study, the combination of the three mentioned techniques is also used to provide new insight about this hypothesis.

Phospholipids with three different chain lengths (DMPC, DPPC and DSPC) and three different headgroups (DPPC, dipalmitoylphosphatidylethanolamine (DPPE) and dipalmitoylphosphatidylserine (DPPS)) are used in the present study. DMPC, DPPC and DSPC have two saturated fatty acid chains with 14, 16 and 18 carbon atoms respectively. As opposed to the work of Maget-Dana and Ptak [9], we use physiological conditions (Tris 10 mM NaCl 150 mM, pH 7.2) in order to be closer to the actual biological systems. In these conditions, DPPE and DPPC are zwitterionic, the polar head of DPPE being smaller than the DPPC one, while DPPS has a net negative charge. Surfactin is partially protonated at physiological pH.

The physico-chemical discussion of our results gives rise to a better understanding of the interaction between surfactin and lipid membrane at a molecular level. Moreover, they give some insight into the molecular mechanisms leading to the biological activity of surfactin. Finally, the work gives a critical view of the