Nanoscale Properties of Mixed Fengycin/Ceramide Monolayers Explored Using Atomic Force Microscopy

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To gain insight into the interactions between fengycin and skin membrane lipids, mixed fengycin/ceramide monolayers were investigated using atomic force microscopy (AFM) (monolayers supported on mica) and surface pressure−area isotherms (monolayers at the air−water interface). AFM topographic images revealed phase separation in mixed monolayers prepared at 20 °C/pH 2 and composed of 0.25 and 0.5 fengycin molar ratios, in the form of two-dimensional (2-D) hexagonal crystalline domains of ceramide surrounded by a fengycin-enriched fluid phase. Surface pressure−area isotherms as well as friction and adhesion AFM images confirmed that the two phases had different molecular orientations: while ceramide formed a highly ordered phase with crystalline chain packing, fengycin exhibited a disordered fluid phase with the peptide ring lying horizontally on the substrate. Increasing the temperature and pH to values corresponding to the skin parameters, i.e., 37 °C/pH 5, was found to dramatically affect the film organization. At low fengycin molar ratio (0.25), the hexagonal ceramide domains transformed into round domains, while at higher ratio (0.5) these were shown to melt into a continuous fengycin/ceramide fluid phase. These observations were directly supported by the thermodynamic analysis (deviation from the additivity rule, excess of free energy) of the monolayer properties at the air−water interface. Accordingly, this study demonstrates that both the environmental conditions (temperature, pH) and fengycin concentration influence the molecular organization of mixed fengycin/ceramide monolayers. We believe that the ability to modulate the formation of 2-D domains in the skin membrane may be an important biological function of fengycin, which should be increasingly investigated in future pharmacological research.

Introduction

Fengycin is a bioactive lipopeptide produced by *Bacillus subtilis* strains that consists of a decapeptide containing a β-hydroxy fatty acid chain (Figure 1A). This class of lipopeptides is composed of closely related variants, which differ both in the length of the fatty acid chain (13−17 carbon atoms) and in the nature of the amino acid in position 6 of the peptide moiety (D-Ala or D-Val, respectively, for fengycin A and fengycin B). Until now, fengycin has not been extensively studied, essentially because of the difficulty of producing and purifying this molecule in large amounts. A few studies have demonstrated that fengycin has a strong surface activity and interesting antifungal property with low hemolytic activity,[1,2] indicating it has a real potential in the pharmaceutical field. The study of its interaction with skin membrane models should therefore provide valuable information in this respect.

The barrier function of the skin is unquestionably attributed to its superficial layer, the stratum corneum (SC).[3−7] This layer consists of keratin-filled corneocytes, organized in a matrix of highly ordered multilamellar lipid sheets. The lipid composition in the SC is unique and plays an essential role in the skin barrier function. Unlike other biological membranes, the SC is almost devoid of phospholipids and its major constituents are ceramides (50% of the lipid mass), cholesterol (25%), and long-chain free fatty acids (10%).[8,9] Nine different classes of ceramides have been identified in the human SC, which are referred to as CER1 to CER9.[10−13] The most abundant type of ceramides observed in epidermal SC is CER2,[9,14−15] which is composed of a sphingosine base to which a nonhydroxy fatty acid is linked (Figure 1B). The fatty acids have a chain length distribution ranging from 16 to 30 carbon atoms, with 24 (C_{24}) and 26 (C_{26}) carbon chain lengths being the most abundant. Because ceramides are known to segregate in domains with negative curvature that affect membrane fission, fusion, and permeability,[16] probing the

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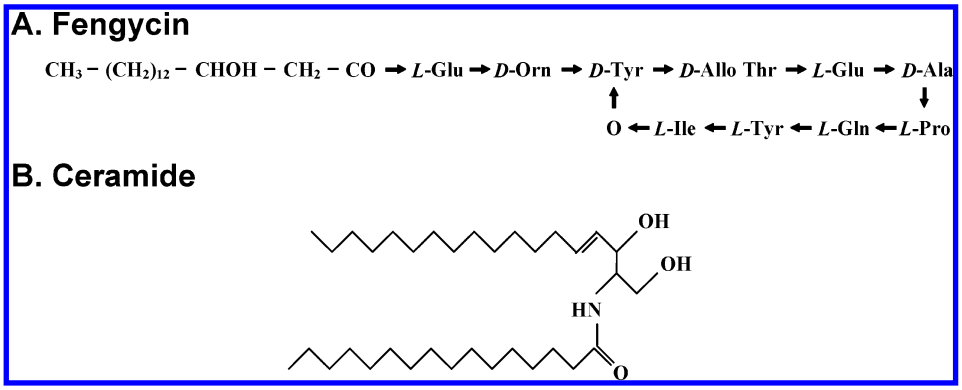

Figure 1. Primary structures of (A) fengycin A (β-hydroxy fatty acid chain of 16 carbon atoms) and (B) ceramide (*N*-palmitoyl-D-sphingosine) used in the present work.

molecular organization and segregation behavior of mixed fengycin/ceramide molecular films is a key to understanding the biological activity of both lipids.

Mixed monolayers at the air−water interface is a well-established approach to study the interactions between different lipids as well as between lipids and other molecules.[17] The advantages of such two-dimensional (2-D) model systems are that they are flat, homogeneous, and stable and allow rigorous thermodynamic analysis. Moreover, parameters such as the nature and the packing of the lipid molecules, the composition of the subphase, and the temperature can be varied in a controlled way. Using the monolayer technique, it is possible to create model membranes that mimic the basic structure composing the multilamellar arrangement of lipids in the stratum corneum and to study the phase behavior of the SC major components.[18−26]

Supported lipid films prepared by the Langmuir−Blodgett (LB) technique or by fusion of lipid vesicles are being increasingly used in biophysical research to investigate the properties of biological membranes and processes such as molecular recognition, enzymatic catalysis, cell adhesion, and membrane fusion.[27−29] They are also attracting considerable interest in applied research for the design of biosensors,[30] biofunctionalization of inorganic solids,[29] crystallization of proteins,[31] and the immobilization of DNA.[32] Supported lipid films offer the possibility to apply surface analysis techniques that could not be used to study real biological membranes, including atomic force microscopy (AFM). In recent years, AFM has emerged as a powerful tool for visualizing lipid domains in phase-separated supported films with nanometer-scale resolution.[33−37] In addition, AFM offers the unique opportunity to probe the nanoscale physical properties and interaction forces of lipid films.[33,38−40]

In this paper, we report on the surface properties of mixed fengycin/ceramide monolayers prepared in different proportions, i.e., fengycin molar ratio going from 0.01 to 0.75, and under two different environmental conditions, i.e., 20 °C/pH 2 and 37 °C/pH 5. The 20 °C/pH 2 conditions are chosen to match previous studies[41−43] and to investigate the properties of fengycin in a protonated state. The 37 °C/pH 5 conditions are more biologically relevant and are suitable to mimic the skin parameters.[44] A ceramide containing a fatty acid with a 16 carbon chain length is selected in order to have a hydrophobic tail with the same length as the acyl chains of the phosphatidylcholine used in previous studies.[41−43] AFM is used to record topographic, friction, and adhesion images of mixed LB monolayers supported on mica, while surface pressure−area isotherms are employed to probe the properties of the films at the air−water interface.

Materials and Methods

Fengycin A with a β-hydroxy fatty acid chain of 16 carbon atoms (molecular weight 1462.8) was produced as described previously (Figure 1A).[45,46] Isolation of this molecule from crude fengycins was performed by preparative reversed phase chromatography using a Vydack 10−15 μm C_{18} column (2.2 × 25 cm, Vydack, Hesperia, CA). The following conditions were used: flow rate of 23 mL/min, acetonitrile/H_2O/TFA 0.05% as mobile phase, under isocratic (48/47/5 v/v/v for 20 min) conditions and linear gradients (51/44/5 v/v/v in 2 min, followed by 0/100/0 v/v/v in 1

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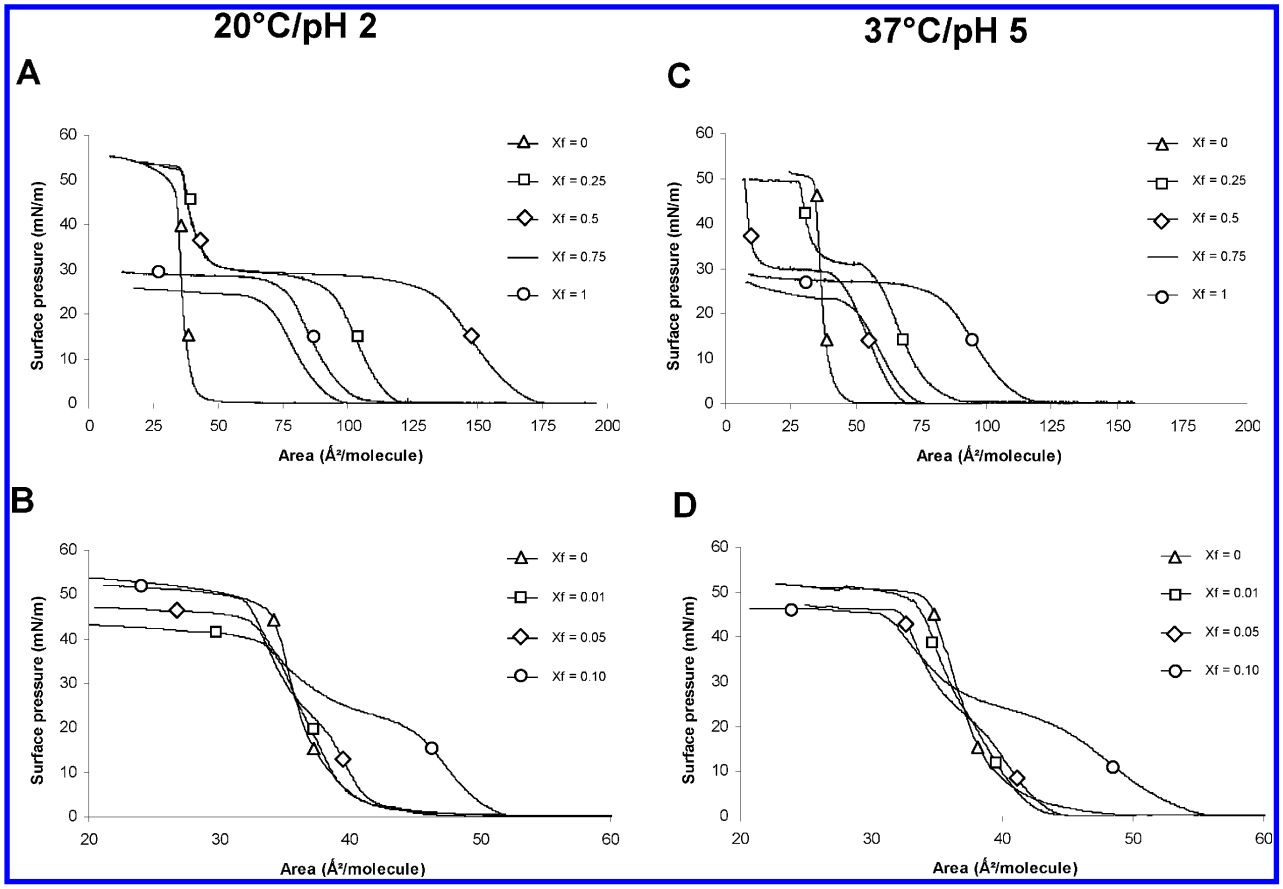

Figure 2. Effect of temperature, pH, and fengycin molar ratio on interfacial properties of mixed fengycin/ceramide monolayers. Surface pressure−area (π−A) isotherms, at the air−water interface, of pure fengycin and ceramide monolayers and of mixed fengycin/ceramide monolayers prepared at high (A, C) and low (B, D) fengycin molar ratios (X_f). The isotherms were recorded at either 20 °C (A, B) or 37 °C (C, D) with an aqueous subphase at pH 2.0 (A, B) or pH 5.0 (C, D). Duplicate experiments using independent preparations yielded similar results.

min), respectively, for eluting the fengycin molecules which were detected at 205 and 214 nm simultaneously. The primary structure and purity of fengycin were ascertained by analytical reversed phase high-performance liquid chromatography (Vydack 10 μm C_{18} column, 0.46 × 25 cm, Vydack, Hesperia, CA), amino acid analysis,[47] and MALDI−TOF mass spectrometry measurements (Ultraflex TOF, Bruckner, Karlsruhe, Germany). The purity of fengycin molecules was always higher than 95%.

LB monolayers were prepared with an automated LB system (KSV Minitrough, KSV Instruments, Helsinki, Finland). *N*-Palmitoyl-D-sphingosine (Figure 1B) was purchased from Sigma (approximately 99% pure). Samples were dissolved in chloroform/methanol (2:1) to give a concentration of 1 mM. Pure solutions as well as 0.01:0.99, 0.05:0.95, 0.1:0.9, 0.25:0.75, 0.5:0.5, and 0.75:0.25 molar mixtures of fengycin and ceramide were spread at 20 ± 0.2 °C on a MilliQ water (Millipore Co., Milford, MA) subphase adjusted to pH 2.0 with HCl or at 37 ± 0.2 °C onto a clean surface of a 5 mM acetate buffer at pH 5.0 in order to mimic skin parameters.[44] Solvents were allowed to evaporate for 15 min before the film was compressed by moving the symmetric barriers at a rate of 10 mm/min. The monolayers were transferred, at 20 and 37 °C, respectively, onto a freshly cleaved mica support at a constant surface pressure of 20 mN/m, i.e., well below the collapse pressure, by vertically raising the support through the air−water interface at a rate of 10 mm/min. The transfer ratios were all close to 1:1. The difference between molecular areas of two independent sets of measurements was less than 5%.

AFM measurements were performed at room temperature (20 °C) using a commercial optical lever microscope (Nanoscope III, Digital Instruments, Santa Barbara, CA). Contact mode topographic and friction images were recorded using oxide-sharpened microfabricated Si_3N_4 cantilevers (Microlevers, Veeco Metrology LLC, Santa Barbara, CA) with a typical radius of curvature of 20 nm and spring constants ranging from 0.01 to 0.1 N/m. The imaging force was kept as low as possible, and the scan rate was 2 Hz. Adhesion maps were created by recording multiple (64 × 64) force−distance curves over defined areas, calculating the adhesion force for each force curve, and displaying the adhesion values as gray levels.

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Results and Discussion

Using the combination of surface pressure−area (π−A) isotherms and AFM, we explored the molecular organization and segregation behavior of mixed fengycin/ceramide monolayers, while varying the environmental conditions (temperature, pH) and fengycin concentration.

Interfacial Properties at the Air−Water Interface. Figure 2 presents the surface pressure−area isotherms obtained at the air−water interface at 20 °C/pH 2 and 37 °C/pH 5 for pure fengycin and ceramide monolayers, and for mixed monolayers at 0.01, 0.05, 0.1, 0.25, 0.5, and 0.75 fengycin molar ratios (X_f). At a surface pressure of 20 mN/m, ceramide and fengycin occupy an area of 37 and 83 Å²/molecule at 20 °C/pH 2, and 38 and 90 Å²/molecule at 37 °C/pH 5.

In both temperature/pH conditions, the π−A isotherms of the pure ceramide show a rapid and sharp increase of the surface pressure when the molecular area decreases. This very low compressibility of the monolayer shows that the ceramide condenses into a close-packed arrangement with a small molecular area implying a vertical chain orientation, even at a very low surface pressure. Löfgren and Pascher have reported surface pressure isotherms for different ceramides showing similar monolayer phase behavior.[18] Monolayers of long-chain fatty acids are also

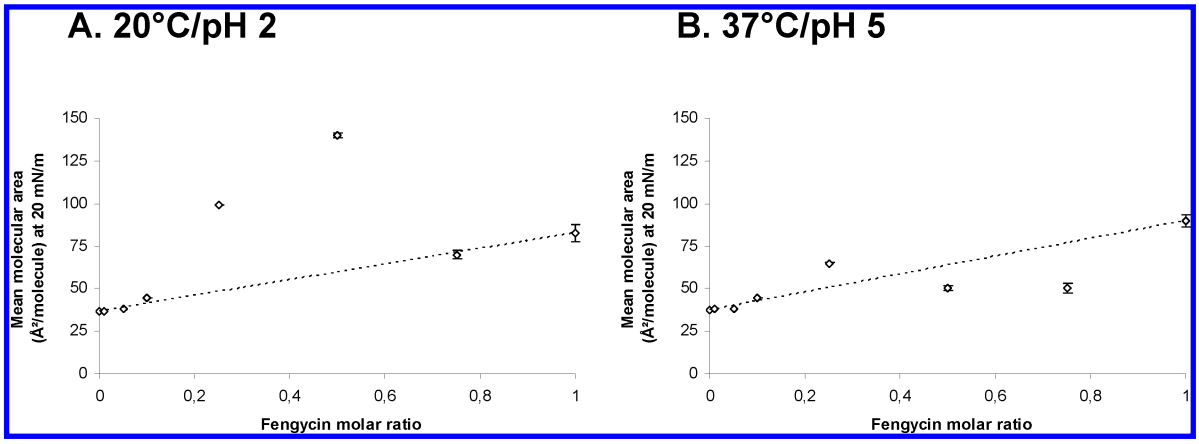

Figure 3. Mean molecular area at a surface pressure of 20 mN/m vs fengycin molar ratio of fengycin/ceramide mixtures. Mean molecular area of monolayers prepared at 20 °C/pH 2 (A) and 37 °C/pH 5 (B). The dashed line represents the additivity rule values. Error bars are the standard errors of the mean.

known to adopt such a dense structure.[48] The main difference between the two sets of environmental conditions is located in the collapse area of the isotherms; i.e., the film collapses at a lower surface pressure (45 versus 50 mN/m) and less abruptly at 20 °C/pH 2 compared to 37 °C/pH 5.

The shape of the $\pi-A$ isotherm of the pure fengycin recorded at 37 °C/pH 5 is similar to that obtained at 20 °C/pH 2. However, increasing the temperature and pH causes an increase of the mean molecular area. Presumably, this expansion of the monolayer results from the appearance of a negative charge on the two glutamic acid residues, which is responsible for electrostatic repulsions between peptide rings of adjacent fengycin molecules, as already reported for surfactin, another lipopeptide with two negative residues.[49]

At 20 mN/m, the surface pressure used for the LB transfer, the molecular organization of ceramide and fengycin in pure monolayers is clearly different, whatever the subphase conditions. While the ceramide monolayer is characterized by a 2-D gel-like organization, fengycin has a 2-D fluid-like organization. Below the gel−liquid crystal transition ($T_m = 95.4$ °C), the ceramide molecules are in a highly ordered gel phase with specific crystalline chain packing.[50] The alkyl chains in this conformation are thought to be extended, rigid, in an all-trans configuration and are probably involved in specific chain−chain interactions with adjacent ceramide molecules. By contrast, the large area occupied by fengycin molecules suggests an organization in which the peptide ring is lying horizontally and the fatty acid chain has no defined orientation, as already suggested for surfactin.[51]

The behavior of mixed monolayers is found to depend dramatically on the environmental conditions, in particular for high fengycin content monolayers: at 37 °C/pH 5, the mean molecular area of mixed monolayers lies between those of pure monolayers, while at 20 °C/pH 2, the area of the mixed monolayers prepared at 0.25 and 0.5 fengycin molar ratios ($X_f = 0.25$ and 0.5) is larger than that of the pure fengycin monolayer. However, when the fengycin content increases ($X_f = 0.75$), the mean molecular area of the mixed monolayer lies between those of pure components whatever the conditions. At 20 °C/pH 2, the compression isotherm is close to that of pure fengycin and presents a similar shape, while at 37 °C/pH 5 the influence of ceramide is still pronounced as the position and the shape of the isotherm are far from that of pure fengycin.

To gain further information about the mixing behavior and the molecular interactions between fengycin and ceramide, thermodynamic analysis were performed according to the procedures described by Maget-Dana[17] and Fang et al.[52] Figure 3 shows the mean molecular area observed for the mixed films at 20 mN/m versus the molar ratio of fengycin. At 20 °C/pH 2, marked positive deviations from the additivity rule are found for the fengycin/ceramide systems at 0.25 and 0.5 fengycin molar ratios (Figure 3A), meaning that fengycin and ceramide molecules have a tendency to form 2-D aggregates in these mixed monolayers. To corroborate these observations, the excess free energy of mixing ΔG_m^{ex} was calculated by the Goodrich relationship (eq 1), where A is the mean molecular area, x is the molar ratio, and subscripts 1, 2, and 12 refer to pure compounds 1 and 2 and their mixtures, respectively.

$$\Delta G_m^{ex} = \int_0^{\Pi} A_{12}\, d\Pi - x_1 \int_0^{\Pi} A_1\, d\Pi - x_2 \int_0^{\Pi} A_2\, d\Pi \qquad (1)$$

Figure 4 shows the plots of the ΔG_m^{ex} values, calculated for a surface pressure of 20 mN/m, as a function of the fengycin molar ratio (X_f). At 20 °C/pH 2 (Figure 4A), a large positive excess of free energy of mixing is observed for $X_f = 0.25$ and 0.5, supporting the formation of domains at the air−water interface. Under the same conditions, mixed monolayers at $X_f = 0.01$, 0.05, 0.1, and 0.75 show an ideal behavior (no significative deviation from the additivity rule and the excess of free energy of mixing ~ 0).

What happens when the temperature and pH are increased? Low fengycin content monolayers ($X_f = 0.01$, 0.05, and 0.10) at 37 °C/pH 5 have the same behavior as those at 20 °C/pH 2; i.e., they follow the additivity rule. At 20 mN/m, the $X_f = 0.25$ monolayer presents a mean molecular area with a smaller positive deviation from the additivity rule and a lower positive excess of free energy of mixing than the same monolayer at 20 °C/pH 2. By contrast, at $X_f = 0.5$ and 0.75 a negative deviation of the mean molecular area from the additivity rule is observed (Figure 3B). This result, together with the negative excess of free energy of mixing (Figure 4B), indicates strong

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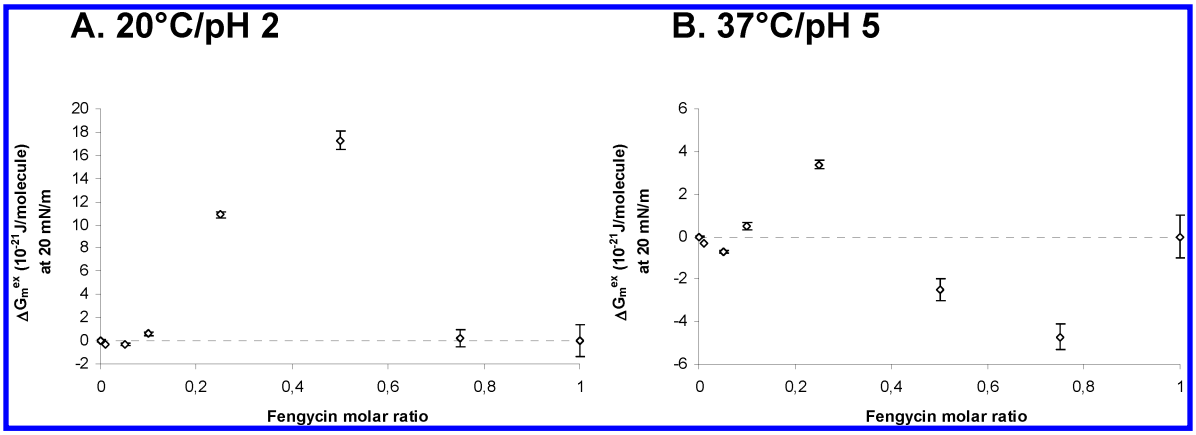

Figure 4. Excess free energy (ΔG_m^{ex}) at a surface pressure of 20 mN/m vs fengycin molar ratio of fengycin/ceramide mixtures. Excess free energy of monolayers prepared at 20 °C/pH 2 (A) and 37 °C/pH 5 (B). The dashed line corresponds to a null excess free energy. Error bars represent the standard deviations. Duplicate experiments using independent preparations yielded similar results.

interactions between the two components at the interface. The very low value of ΔG_m^{ex} suggests the formation of a complex between fengycin and ceramide.[17,53]

Nanoscale Properties of Supported Monolayers. Mixed fengycin/ceramide monolayers prepared at X_f = 0.25 and 0.5 were transferred on mica and analyzed by AFM in view of their strikingly different interfacial behavior: for the $X_f = 0.25$ monolayer, both the deviation from the additivity rule and the excess of free energy of mixing were positive whatever the experimental conditions, while for $X_f = 0.5$, they became negative at 37 °C/pH 5.

Parts A, B, and C of Figure 5 present AFM topographic, friction, and adhesion images obtained for a mixed fengycin/ceramide monolayer at $X_f = 0.25$. Phase separation is clearly observed in all images in the form of hexagonal domains ~200−600 nm in size covering about 50% of the surface and embedded in a continuous matrix. The phase separation observed by AFM is consistent with the properties of the monolayer at the air−water interface, which suggests the formation of 2-D domains (Figures 3A and 4A). In light of the surface pressure−area isotherms, we attribute the lower and the higher levels in the topographic images to fengycin- and ceramide-enriched phases, respectively. Indeed, the area fraction occupied by the two phases may be compared with that expected for fengycin and ceramide in the mixed monolayer at the air−water interface. Considering the fengycin molar ratio (X_f) and the areas occupied by the fengycin and ceramide molecules in pure monolayers (A_f and A_c), the fraction of the surface occupied by fengycin in mixed monolayers at the air−water interface (γ) may be calculated as follows:

$$\gamma = X_f A_f / (X_f A_f + (1 - X_f) A_c) \qquad (2)$$

At 20 mN/m and $X_f = 0.25$, a γ value of 0.44 is obtained, which agrees well with the 50% surface coverage observed in the AFM images. This leads us to believe that the hexagonal domain structures correspond to 2-D crystals of ceramide molecules standing perpendicular on the surface and organized with a 6-fold symmetry, while the surrounding phase would essentially consist of fengycin in the fluid phase. This difference in phase organization is directly supported by the 0.9 ± 0.1 nm step height measured between the two phases in the topographic image: while ceramide molecules are vertically oriented

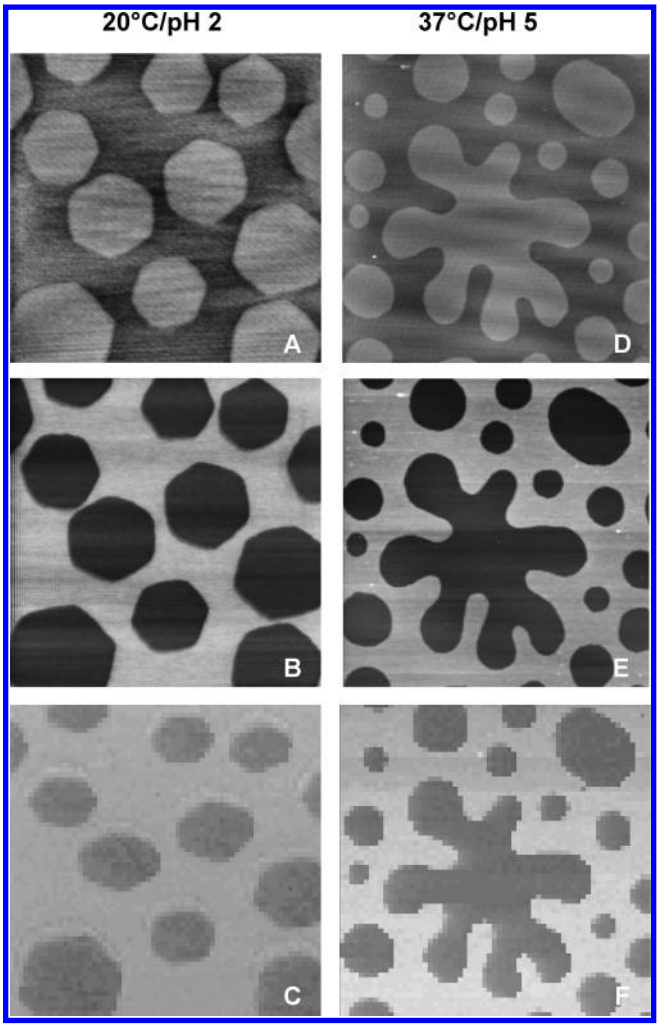

Figure 5. Effect of temperature and pH on nanoscale organization of mixed ceramide/fengycin monolayers. AFM images obtained for mixed monolayers prepared at 0.25 fengycin molar ratio and 20 °C/pH 2 (A−C) or 37 °C/pH 5 (D−F): (A, D) topography [z-range 5 (A) and 10 nm (D)]; (B, E) friction; (C, F) adhesion force [z-range 20 (C) and 10 nN (F)]. The image size is 2 μm × 2 μm (A−C) and 15 μm × 15 μm (D−F). Lighter levels in the images correspond to higher height, higher friction, and higher adhesion. Duplicate experiments using independent preparations yielded similar results.

and in a 2-D gel-like organization, fengycin molecules are probably lying horizontally and yielding a 2-D fluid-like organization. As already shown for other monolayer

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systems,[33,35,36] the measured step height would result both from a difference in the orientation of the molecules and in the relative mechanical properties of the two phases. The hexagonal ceramide texture may actually reflect a "hexatic" phase, which in essence consists of 2-D liquid crystals that lack transitional order but retain long-range orientational order.[54] It may also be related to the well-known ability of ceramide to favor hexagonal II phase formation by segregating in domains with negative curvature.[16] Interestingly, we note that AFM images of phase-separated fengycin/dipalmitoylphosphatidylcholine prepared under the same conditions (temperature, pH, X_f) did not show such 2-D hexagonal crystalline domains, confirming that this texture is specific to ceramide (unpublished data).

The finding that ceramide can form crystalline domains is consistent with previous AFM studies. Similar hexagonal liquid condensed domains were observed by Chi and co-workers in stearic acid monolayers.[55] Moreover, Ekelund et al. showed that 2-D ceramide crystals are formed in mixed ceramide monolayers.[21] However, the authors always observed rectangular domains, suggesting that these are of different nature than those reported here. In future work, it would be most interesting to assess to what extent 2-D ceramide crystals occur in natural biological membranes and what specific biological roles they may play.

The friction and adhesion contrasts provide further evidence that ceramide and fengycin phases have different molecular orientations. Compared to the closely packed ceramide domains,[18] the molecular disorder of the fengycin molecules is expected to give rise to a larger tip−sample contact area, and therefore to larger friction and adhesion in the film.[33,56] Similar observations were made for surfactin in friction images.[41] However, in the case of surfactin, the adhesion images were not contrasted, suggesting that the packing properties of surfactin and fengycin monolayers are different. In fact, these data suggest that fengycin would be less organized than surfactin, an hypothesis that is directly confirmed by the $\pi-A$ isotherm of the two lipopeptides: while surfactin exhibits a liquid-expanded to liquid-condensed state transition,[41] the $\pi-A$ isotherm of pure fengycin presents no sharp increase of surface pressure at very low areas per molecule, indicating the absence of liquid-condensed and solid states and thus a tendency to have a more random orientation than surfactin. Moreover, the two-dimensional compressibility of the pure lipopeptide monolayers, calculated by eq 3, is lower for surfactin ($C_s = 7.64$ $N^{-1}\cdot m$) than for fengycin (11.22 $N^{-1}\cdot m$), suggesting that the surfactin monolayer presents a lower interfacial elasticity and, therefore, is more organized than the fengycin monolayer.

$$C_s = (-1/A)(dA/d\pi) \quad (3)$$

A striking finding of this study is that the domain shape changes dramatically with the experimental conditions. In Figure 5D−F it can be seen that the hexagonal forms observed at 20 °C/pH 2 transform into small round domains together with large flower-like domains at 37 °C/pH 5. This suggests that increasing temperature and pH causes melting of the crystalline ceramide domains into noncrystalline domains. There are two other arguments supporting this view. First, the step height measured between the two phases is significantly smaller, i.e., 0.6 ± 0.1 nm, suggesting that the ceramide molecular configuration has changed: at 37 °C/pH 5, the ceramide molecules would no longer be perfectly vertically oriented but would be slightly disorganized and tilted, giving rise to a smaller film thickness. Second, the surface pressure−area isotherms point to an increase of miscibility at 37 °C/pH 5: both the positive deviation from the additivity rule and excess of free energy decrease when comparing 20 °C/pH 2 with 37 °C/pH 5, suggesting the two compounds are more miscible.

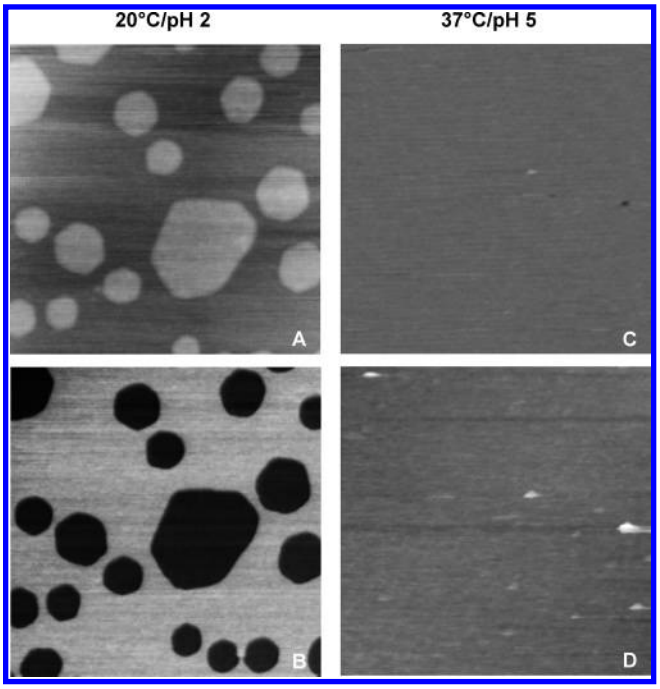

Figure 6. Effect of fengycin molar ratio on nanoscale organization of mixed ceramide/fengycin monolayers. AFM images obtained for mixed monolayers prepared at 0.5 fengycin molar ratio and 20 °C/pH 2 (A, B) or 37 °C/pH 5 (C, D): (A, C) topography (z-range 10 nm); (B, D) friction. The image size is 2 μm × 2 μm.

The change in domain shape is likely to originate from a difference in the molecular interactions within the film. The observation of complex, elongated domain shapes reflects the competition between line tension and long-range dipole interactions.[57−59] Circular shapes are favored when the line tension of the interface between the two lipid phases dominates, while distorted, elongated structures are favored when the electrostatic dipole−dipole repulsion between the molecular dipoles associated with the lipid molecules becomes important.

Finally, we note that the fengycin molar ratio has a strong influence on the nanoscale properties of the monolayers. Figure 6 presents AFM topographic and friction images obtained for mixed fengycin/ceramide monolayers at 0.5 fengycin molar ratio. At 20 °C/pH 2, phase separation is observed in the form of hexagonal domains embedded in a continuous matrix, giving rise to friction (Figure 6B) contrast. As expected, the surface coverage of the ceramide domain is smaller than that observed at $X_f = 0.25$. Strikingly, at 37 °C/pH 5 (Figure 6C,D), the film appears to be uniform, not only in the topographic image but also in the friction image, leading

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us to believe that the ceramide domains have melted into a fluid phase that has subsequently mixed the fengycin phase. This observation is in excellent agreement with the thermodynamic analysis of the monolayer properties at the air−water interface which supports the formation of a complex between fengycin and ceramide under these conditions. Further work is needed to establish whether the correlation between the formation or absence of domains found with AFM and the sign of the excess free energy of mixing is valid at all fengycin fractions.

Accordingly, these results indicate that not only the environmental conditions (temperature, pH) but also the fengycin concentration influence the tendency of ceramide to form 2-D domains. These findings may be of great biological and medical relevance. Ceramide is known to promote lateral phase separation in bilayers[60,61] and is thought to play an important role in membrane destabilization and fusion.[16,62] We expect that an important function of fengycin may be to modulate the ceramide properties in the skin membrane (molecular organization, phase separation, fusogenic activity). Therefore, we also anticipate that the remarkable properties of fengycin will be increasingly studied and exploited in pharmaceutical research. In future studies, it would be interesting to investigate mixed monolayers containing not only ceramides but also free fatty acids and cholesterol to better mimic the skin membrane composition. Another exciting direction for further research would be to study the incorporation of lipopeptides in monolayers from the subphase of a Langmuir trough.

Conclusions

Although the interactions of biologically active lipopeptides with lipids have been extensively studied, there is no information available concerning fengycin. In this study, we show that both the environmental conditions (temperature, pH) and fengycin concentration influence the molecular organization of mixed fengycin/ceramide monolayers. At 20 °C/pH 2, AFM reveals phase separation in mixed monolayers for both 0.25 and 0.5 fengycin molar ratios (X_f), in the form of 2-D hexagonal crystalline domains of ceramide surrounded by a fengycin-enriched fluid phase. This is consistent with the contrast of friction and adhesion AFM images as well as with the monolayer properties at the air−water interface that point to the formation of 2-D domains under these conditions.

Increasing the temperature and pH to values corresponding to the skin parameters (37 °C/pH 5) dramatically affects the film organization: at $X_f = 0.25$, the hexagonal ceramide domains transform into round domains, while at $X_f = 0.5$ they change into a continuous ceramide/fengycin fluid phase. Consistent with this, the monolayer properties at the air−water interface support the formation of complexes between individual fengycin and ceramide molecules. These results indicate that fengycin is a natural surface active agent with strong membrane activity, thereby confirming its potential in pharmacology.

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