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Aggregation behavior and surface morphology studies of surfactin in Langmuir–Blodgett films

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ABSTRACT

Surfactin monolayer, which was transferred from the air–water interface onto mica substrate by Langmuir–Blodgett (LB) technique, was characterized with atomic force microscopy (AFM). The effect of deposition pressure on morphologies of SuC14 (surfactin analogue with a β-hydroxyl fatty acid chain of 14 carbon atoms) LB films was investigated. AFM topographic image reveals phase separation at lower deposition pressure, indicating the existence of surfactin domains. At higher deposition pressure, the mica substrate was fully covered with surfactin monolayer, and spherical aggregates were formed atop the monolayer. Surfactin analogues (same polar head, different alkyl chain length) were applied to study the influence of the hydrophobic chain length on the aggregation behavior. On the basis of Fourier transform infrared (FTIR) measurements, the hydrogen bonding is supposed to be involved in the aggregates. These results, concerning the aggregates formation in surfactin LB films, provide new insights into the interfacial behavior of the amphiphilic lipopeptide at the air–water interface.

1. Introduction

Surfactin, produced by *Bacillus subtilis* [1], is a representative family of lipopeptides for its exceptional surfactant power since it lowers the surface tension of distilled water from 72 mN m^{-1} to 27 mN m^{-1} at a concentration as low as 20 μM [2]. Furthermore, it possesses specific biological properties such as antiviral, cytolytic and hemolytic activities [3–5].

The hydrophilic segment in surfactin molecule consists of a peptide loop of seven amino acids, and a C13–C15 fatty acid chain on the other end is hydrophobic (shown in Fig. 1). The amphiphilic character of surfactin molecules would exhibit a fascinating version of molecular organization at interfaces. Recently, much attention has been paid to the structure of surfactin and its interfacial behavior for the fact that surface activities and biological properties mostly occur at interfaces [6,7]. Several authors have experimentally investigated molecular organization of surfactin at the interface. Maget-Dana and Ptak [8] studied the surface pressure–area isotherm of surfactin, suggesting several molecular conformations in the monolayer. Ishigami et al. [9] proposed that surfactin molecules would display dimer formation between two surfactin molecules at the air–water interface. Deleu et al. [10] investigated a mixed monolayer of surfactin and DPPC, indicating a different molecular orientation between surfactin and DPPC at the air–water interface. Eeman et al. [11] recently examined the interfacial behavior of cyclic surfactin analogues and linear surfactin. Moreover, Gallet et al. [7] computationally simulated surfactin conformation of a most stable molecule structure and a seven molecules' assemblage at a hydrophobic/hydrophilic interface.

The phenomenon of amphiphile self-association had been suggested by Langmuir [12], and the formation of novel surface micelle has been reported for many systems [13–16], such as diblock copolymers, fluorinated long-chain acids, and semifluorinated alkanes. The tendency of surfactin to self-associate and form bi-dimensional aggregates was formerly proposed by some researchers [9,10,17]. In our previous study [18], it appears that surfactin molecules in Langmuir monolayer could self-assembly into nanometer size clusters. Aggregation behavior of surfactin involved in the formation of such type clusters is believed to be novel and interesting for further studying.

The aim of this paper was to study the aggregation behavior of surfactin in Langmuir monolayer with the combination of AFM and LB technique. Three purified surfactin analogues with β-hydroxyl fatty acid chain of 13–15 carbon atoms are used to evaluate the effect of hydrophobic chain length on the aggregation behavior. Furthermore, the Fourier transform infrared (FTIR) spectroscopy is applied to get a better understanding of interaction forces involved in the aggregates formation.

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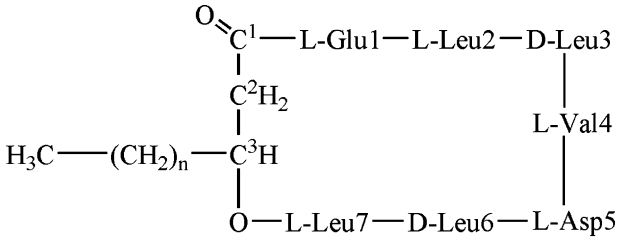

Fig. 1. Primary structure of surfactin (a peptide loop containing seven amino acid residues bonds to a β-hydroxyl fatty acid chain, n = 9 to 11 denotes β-hydroxyl fatty acid chain with carbon atoms ranging from 13 to 15).

2. Experimental details

2.1. Materials

Surfactin molecules used in the experiment were originally separated from the cell-free broth of *Bacillus subtilis* HSO121 [19]. The isolation, purification and structural analysis were conducted in our laboratory [20]. Surfactin analogues with a β-hydroxyl fatty acid chain of 13–15 carbon atoms (denoted as the same name of SuC13, SuC14, SuC15 as in Ref. [7]), of which the primary structure is shown in Fig. 1, were collected by semipreparative reversed-phase high-performance liquid chromatography (HPLC), the structural analysis was performed by TOF MS/MS, GC–MS combined with amino acid analysis and analytical HPLC [20,21].

2.2. Compression isotherm and deposition of LB films

The methods for measuring compression isotherms and preparing samples for AFM observations were previously reported [18], but explained here briefly. A stock solution at a concentration of 1 mM was obtained by dissolving surfactin into hexane and chloroform with a ratio of 2:1 (v/v). Surfactin monolayer was prepared on a Langmuir–Blodgett trough (612D, Nima Technology, England) by spreading 30 μL of the stock solution onto purified water (18 MΩ cm, pH ~6). The monolayer was maintained without compression for 15 min to ensure the complete evaporation of the solvents. Monolayers were then deposited at a constant pressure, by raising mica substrate vertically through the air–water interface at 2 mm min^{-1}. All compression isotherms were recorded at a barrier speed of 0.1 nm^2 min^{-1} $molecule^{-1}$. The subphase temperature was controlled by a water bath. Each isotherm was performed three times and had a good reproducibility.

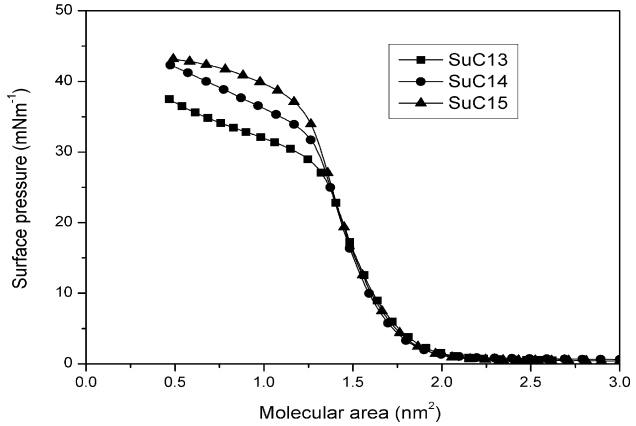

Fig. 2. Surface pressure–molecular area (π–A) isotherms of surfactin analogues on pure water (pH ~6) at 25 °C.

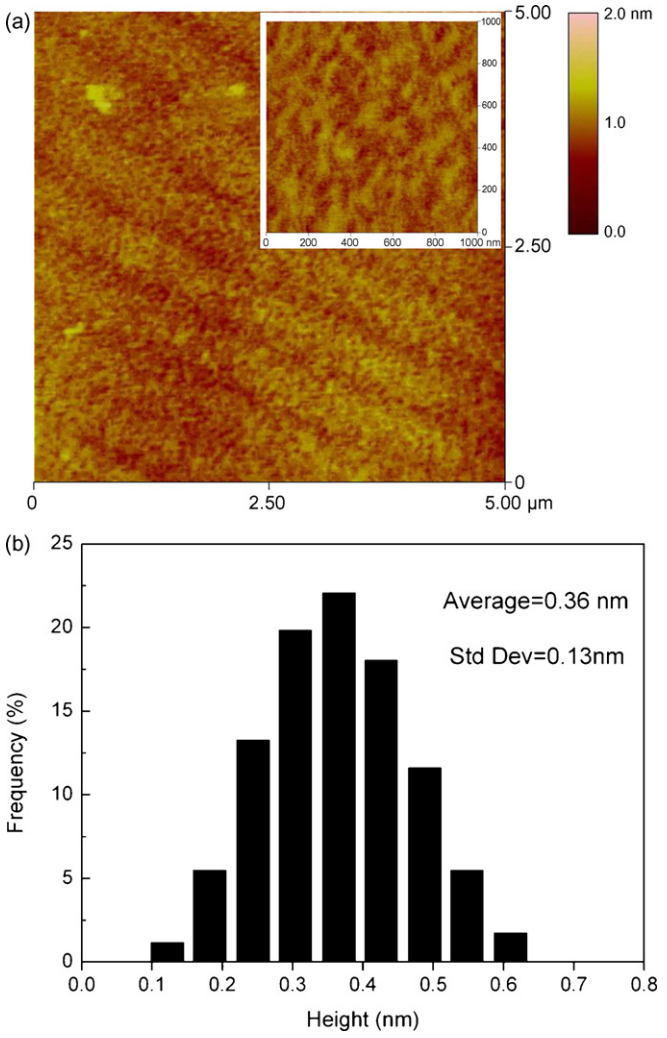

Fig. 3. (A) Top view AFM image of SuC14 LB film deposited at 0.5 mN m^{-1}. Inset: magnified AFM image for clarity. The sizes of these images are 5 μm × 5 μm and 1 μm × 1 μm in lateral, 2 nm in vertical. (B) Height distribution of SuC14 domains observed in the AFM image.

Especially 10-layer LB film was prepared for FTIR measurement. Initially, the mica substrate was submerged in the water subphase and the first monolayer was transferred during an up-stroke at a constant pressure. The water surface was then cleared and the mica was subsequently submerged in the water subphase, followed by dropping the spreading solution and transferring the second layer at the constant pressure. Repeatedly, the third layer, the forth layer, etc. were successfully transferred. Finally, the Z-type 10-layer LB film was obtained. The deposited single-layer and 10-layer LB films were purged under dry air before AFM characterization and FTIR measurement.

2.3. AFM images

AFM (AJ III, Aijian Nanotechnology, China) was used to observe the surface morphologies of surfactin LB films. All AFM measurements were performed at room temperature (20 °C) in tapping mode, using silicon cantilever (Mikromasch Company, Estonia) with a resonance frequency in the range of 240–400 kHz, and a spring constant of 48 N m^{-1}. AFM images were obtained with a maximum scan range of 18 × 18 μm^2 and the scan rate was 1–2 Hz. All images were gained from at least three macroscopically sepa-