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Molecular orientation and film structure of gramicidin on highly oriented pyrolitic graphite

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ABSTRACT

Gramicidin molecules were deposited on HOPG surfaces to characterize molecular orientation and film structure as a function of surface coverage and temperature. At low coverage (0.35 ML), the molecules adopted a flat-lying orientation and formed dendritic islands. At higher coverage (0.86 ML), molecules adopted an upright orientation and circular holes formed in the films. The upright film exhibited higher adhesion in force spectroscopy measurements, supporting our molecular orientation assignments. At elevated deposition temperatures (50 °C) on the higher coverage films, the holes were still present, but partially filled in. At 60 °C the film structure was quite different, forming tall irregular islands without the circular holes observed at lower temperatures. These results demonstrate that gramicidin molecular orientation and film structure on HOPG can be controlled by tuning the surface coverage and deposition temperature.

1. Introduction

The "bottom up" approach to biomolecular assembly can be exploited to fabricate a variety of 3D nanostructures, and the scale of such structures can be extended to micrometer dimensions [1,2]. Hence, numerous applications have evolved that utilize these strategies, such as bioscaffolds [3–5], nanoreactors [6,7], nanocarriers [8–11], and nanotemplates [12–15]. If such assembly processes can be controlled by tuning the molecular structure and environmental conditions, then large scale fabrication can be accomplished, spanning nanometer to micrometer length scales. Further, if the mechanism for self-assembly can be better understood, one can gain control over the types of nanostructures that can be formed.

Gramicidin is a linear ion-channel peptide that exhibits antibiotic activity [16–19]. The outer surface of gramicidin is hydrophobic while the internal ion channel is hydrophilic. The ends of the molecules are slightly hydrophilic due to the presence of amino and carboxyl groups. These amphiphilic properties make gramicidin a promising biomolecular building block for creating nanostructures on surfaces and for characterizing molecular assembly and film formation. In this study, we show that surface coverage and substrate temperature can be tuned to control the molecular orientation and film structure of gramicidin on HOPG.

2. Materials and methods

Gramicidin from *Bacillus brevis* (Sigma) was used for all experiments. Gramicidin has the amino acid sequence HCO–L-Val^{1}–Gly^{2}–L-Ala^{3}–D-Leu^{4}–L-Ala^{5}–D-Val^{6}–L-Val^{7}–D-Val^{8}–L-Trp^{9}–D-Leu^{10}–L-Xxx^{11}–D-Leu^{12}–L-Trp^{13}–D-Leu^{14}–L-Trp^{15}–NHCH2CH2OH, where Xxx^{11} is Trp in gramicidin A, Phe in gramicidin B, and Tyr in gramicidin C. The commercial form of gramicidin is a mixture of the three components A:B:C in the ratio of 80:4:16. At least two different dimer conformations are known to form, a double helix and a helical dimer, though no attempt to identify these was made in this study [16,18–20]. A solution of 100 ppm gramicidin in methanol (GC grade, Echo Chemical Co. Ltd.) was prepared and deposited by droplet onto freshly cleaved HOPG surfaces (SPI Incorporated, West Chester, PA). Methanol evaporated within minutes and the films were immediately characterized using AFM (Asylum Research, MFP-3D) in noncontact mode or by performing force measurements. Total deposition amounts of gramicidin of 84, 168, 252, and 336 pmol were used, though only the 84 and 336 pmol data are shown in the paper. Please refer to the Supplemental section for the 252 pmol image and description. For thermal studies, samples were heated using a hot plate and allowed to equilibrate for several minutes at each temperature prior to depositing gramicidin. Local relative film coverage was estimated from the AFM images.

3. Results and discussion

In Langmuir Blodgett (LB) studies, a fixed amount of amphiphilic molecules is deposited on water, then the area is reduced

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mechanically to increase the surface pressure and molecular packing density. For gramicidin molecules present at the air/water interface, it was shown that at higher pressures, gramicidin molecules formed dimers in a close packed structure [21,22]. To simulate this controlled surface tension technique on HOPG samples, we varied the total amount of gramicidin deposited on a 1 cm^2 area of the surface. Methanol was used as the solvent because it wets the HOPG surface well, dissolves gramicidin completely, and evaporates rapidly under ambient conditions ensuring an even distribution of gramicidin on HOPG.

Fig. 1a shows a topographical AFM image ($1 \times 1\ \mu m^2$) of a film prepared by depositing 84 pmol of gramicidin over a 1-cm area of a freshly cleaved HOPG surface and allowing the solvent to evaporate. The relative gramicidin coverage was 35% as determined from the AFM image. At this coverage, the molecules lie very flat on the surface and form 2D dendritic islands. The height of the film is ~0.22 nm as shown in the lower line scan of Fig. 1c. The upper line scan shows a cross section across an HOPG step (~0.34 nm) [23,24], which provides an internal height calibration.

The diameter of gramicidin molecules is ~1.6 nm based on X-ray crystallography results and on calculations [17,20,25]. Compared to the measured height of the gramicidin films shown in Fig. 1c, this amounts to a flattening of the molecules by a factor of >7. Tsukruk et al. observed significant flattening of dendritic macromolecules on SiO_2 surfaces [26]. In that case, they suggested that the dendrimers were likely both collapsed and highly compressed along the surface normal. In this experiment, we attribute the flattening of gramicidin molecules in this low coverage film to a strong hydrophobic interaction between the outer surface of the gramicidin molecules and the HOPG surface [25–29] and to a corresponding flattening (collapse and compression) of the molecules against the surface.

When the deposited amount of gramicidin was increased to 336 pmol, a distinct change in the film structure was observed as shown in Fig. 1b. The relative coverage for this film was estimated at 88% and the film exhibited homogeneously dispersed "holes" of exposed HOPG (148 ± 10 nm – calculated from >120 holes). A line scan across one of the holes in Fig. 1d shows that the molecular film thickness of the ring around the hole is ~2.8 nm. This is consistent with the calculated length of the gramicidin monomer (2.6–3.0 nm) [17,20,25].

We have considered two possibilities to account for observed holes in the gramicidin films. Initially, we considered a solvent drying effect, whereby molecules are dragged to the outer surface of drying droplets and then deposit on the surface creating rings. However, no such effects were observed on lower coverage films even up to 252 pmol depositions (Supplemental section). Further, the surrounding film did not exhibit any other features or structures that would suggest a drying effect deposition model. The blue circles overlaid on one of the ring structures in Fig. 1b illustrate a second possible model to describe the origin of the holes. Diociaiuti et al. proposed that gramicidin formed hexomeric aggregate structures in phospholipid membranes [30]. These were suggested to further form second and third level fractal aggregates which accounted for the large ring structures observed in that study. A ring of 12 second-level fractal aggregate structures, as depicted by the blue circles, could account for the ringed holes observed in the high coverage gramicidin film shown in Fig. 1b.

These results may be explained by applying a micelle model. At low concentrations, gramicidin molecules behave independently, while at higher concentrations, they aggregate to form micelle-like structures. In LB films a critical micelle concentration is attained when the surface tension reaches a minimum. Any addition of surfactant molecules beyond this point goes into micelle formation

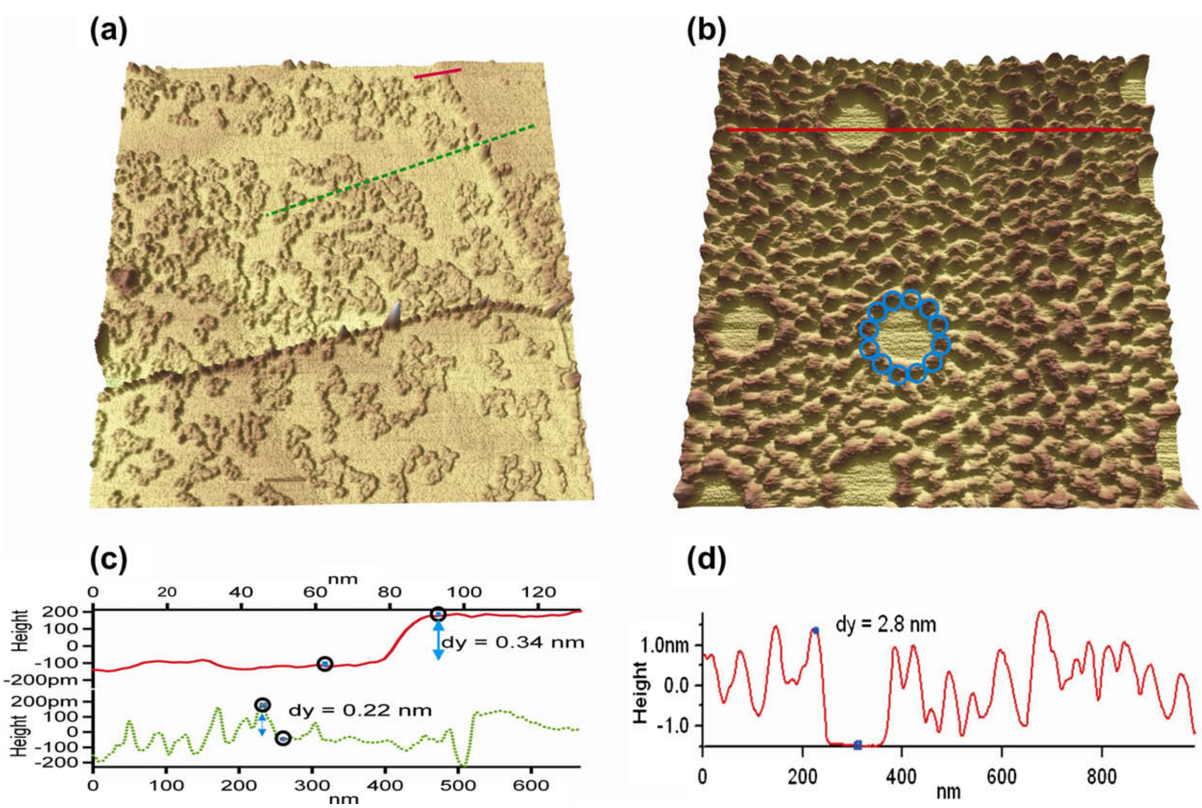

Fig. 1. Gramicidin films on HOPG. For an initial deposition of 84 pmol: (a) flat-lying dendritic islands are formed, while at 336 pmol, (b) films composed of upright gramicidin molecules with circular holes in the film were observed. The film height of the flat-lying film was ~0.22 nm (c). The height of the rings around the holes in the upright film was ~2.8 nm (d). The blue circles on (b) are discussed in the text, and describe a possible model for the rings in (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)