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Electrostatic interactions of colicin E1 with the surface of *Escherichia coli* total lipid

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Abstract

The surface properties of colicin E1, a 522-amino acid protein, and its interaction with monolayers of *Escherichia coli* (*E. coli*) total lipid and 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DOPC) were studied using the Langmuir–Blodgett (LB) technique. Colicin E1 is amphiphilic, forming a protein monolayer at the air/buffer interface. The protein is thought to interact with the *E. coli* total lipid head groups through electrostatic interactions, followed by its insertion into the lipid monolayers. Supported lipid bilayers (SLBs) of *E. coli* total lipid and DOPC, deposited onto mica at the cell membrane equivalence pressure for *E. coli* and incubated with colicin E1, were imaged by contact mode atomic force microscopy (CM-AFM). Colicin E1 formed protein aggregates on DOPC SLBs, while *E. coli* total lipid SLB was deformed following its incubation with colicin E1. Corresponding lateral force images, along with electrostatic surface potentials for colicin E1 P190, imply a direct interaction of colicin E1 with lipid head groups facilitating their charge neutralization.

Keywords: Atomic force microscopy; Supported planar bilayer; Colicin E1; Cytotoxin; DOPC; *Escherichia coli* total lipid; Langmuir–Blodgett; Monolayer; Protein–Lipid Interaction

1. Introduction

Proteins and lipids are commonly colocalized in natural systems making their mechanism of interaction a topic of great interest [1]. Recent experimental and theoretical studies show that not only do physical interactions between lipids and proteins affect lipid arrangements, but also the physical properties of neighboring phospholipids can influence protein activity [2,3]. The latter idea is supported by the regulation of protein activity as a function of its localization [4] and relative lipid composition.

Colicin E1, is a 522 amino acid bactericidal protein that is cytotoxic to susceptible cells through its ability to form voltage gated, ion-conductive channels within the cytoplasmic membrane. The functional domains of colicin E1, namely translocation (T; 1–199), receptor-binding (R; 232–337), and channel-forming (C; 372–522), are joined by linker regions 200–231 and 338–371, respectively [5]. Subsequent to receptor binding, where the vitamin B12 receptor is the primary receptor of the E-colicins, the C domain must be translocated across the outer membrane of the periplasmic space. The C domain then associates with the cytoplasmic membrane followed by its insertion into the bilayer. The membrane-associated and transmembrane states of colicin E1 have been studied extensively by Cramer and co-workers [6–9], leading to a model in which the C domain interacts with lipid membranes initially through electrostatic interactions between the positive charges of the protein and the negatively charged membrane surface, followed by a rapid transition to non-electrostatic binding without disruption of the bilayer's integrity. The structure for the C-terminal polypeptide of the channel, P190, has been solved to 2.5 Å [10] and P190 has been shown to both bind to membranes and to form channels in membranes. Acidic pH will induce colicin E1 binding and its insertion into the membrane.

Abbreviations: AFM, atomic force microscopy; CL, cardiolipin; CM, contact mode; DOPC, 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine; LB, Langmuir–Blodgett; LF, lateral force; PBS, phosphate buffer solution; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SLB, supported lipid bilayer

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SLBs, especially those prepared by the Langmuir–Blodgett (LB) technique and vesicle deposition, have been extensively studied as biomimetics of biological lipid membranes [11–13]. Recently, atomic force microscopy (AFM) has been applied to the study of biological systems, including the domain structure of SLBs and the effect of incorporating small amounts of protein therein [14,15].

Colicin E1 is composed of 10 alpha helices, capable of forming a transmembrane domain [16], but it is also soluble in polar solvents [6]. Therefore, it was appropriate to investigate whether colicin E1 is capable of forming a monolayer at an air/buffer interface. Herein, we present a study of the properties of colicin E1 at the air/buffer interface and its interactions with *E. coli* total lipid and DOPC monolayers at the air/buffer interface, where DOPC serves as a zwitterionic reference system. AFM reveals the morphology of SLBs prepared from *E. coli* total lipid at a series of surface pressures using the LB method, and following its incubation with colicin E1 for an SLB prepared at the cell membrane equivalence pressure. AFM images of *E. coli* total lipid and DOPC SLBs incubated with colicin E1, in conjunction with an electrostatic surface analysis of colicin P190, provide direct evidence for the charge neutralization of the *E. coli* total lipid bilayer surface by colicin E1.

2. Materials and methods

2.1. Materials

E. coli total lipid extract (25 mg/mL in chloroform) and 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC, 25 mg/mL in chloroform) were purchased from Avanti Polar Lipids (Alabaster, AL), diluted (1 mg/mL) in chloroform and sonicated (30 min) in an ice bath prior to use. Colicin E1 was a kind gift from Drs. Stanislav D. Zakharov and William A. Cramer (Biological Sciences, Purdue University). Nanopure (Barnstead, USA) water (resistivity ≥ 18 MΩ cm^{-1}, pH = 5.5 and surface tension = 71 mN/m) was used for all studies. PELCO mica sheets (Ted Pella Inc., Redding, CA) were cut (1×2 cm^2) and freshly cleaved prior to bilayer deposition. Chloroform (purity 99.8%, spectrophotometric grade) and all other chemicals used were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Protein adsorption onto the air/buffer interface and lipid monolayer

The adsorption of colicin E1 onto the air/buffer interface was studied using a commercially available Langmuir–Blodgett (LB) trough (Model 611, NIMA technology, Coventry, England), for which the surface pressure was measured with a Wilhelmy balance. Changes in surface pressure caused by the adsorption of protein at the interface were recorded as a function of time (π–t isotherms) following the injection of a small volume (5, 10, 15, 25, 40 and 60 μL) of colicin E1 (10 mg/mL, 174.6 μM) in phosphate buffer solution (PBS, 0.1 M, pH = 7.0) into the PBS subphase (150 mL). All LB experiments were recorded at room temperature.

E. coli total lipid chloroform solution (20 μL, 1 mg/mL) was dropped onto the PBS subphase, the chloroform allowed to evaporate (10 min) and the lipid monolayer that formed was compressed to 32 mN/m following two rounds of annealing. Colicin E1 (10 μL, 10 mg/mL in PBS) was then injected into the PBS subphase and the π–t isotherm was recorded until the surface pressure remained constant. The bar speed (5 mm^2/min) was the same for all experiments, and monolayers were annealed by compression–expansion cycles until both the compression and expansion isotherms were identical to the previous ones.

The interaction of colicin E1 with the DOPC monolayer at the air/buffer interface was used as a reference system following the same procedure as for *E. coli* total lipid. Briefly, the DOPC solution (20 μL, 1 mg/mL in chloroform) was dropped onto the PBS subphase, the chloroform evaporated, the monolayer was compressed to a surface pressure of 32 mN/m, the protein (10 μL, 10 mg/mL in PBS) injected into the PBS subphase, and the π–t isotherm recorded until the surface pressure remained constant (~20 min).

2.3. π–A isotherms of *E. coli* total lipid, colicin E1 and mixtures thereof

A solution of *E. coli* total lipid (35 μL, 1 mg/mL in chloroform) was spread onto a PBS subphase (150 mL) followed by a 10 min waiting period. The monolayer was compressed and the surface pressure–area (π–A) isotherm of *E. coli* total lipid was recorded. The π–A isotherm of colicin E1 was recorded by injecting protein (5 or 10 μL, 10 mg/mL in PBS) into the PBS subphase, incubating until the surface pressure remained constant (~20 min) to account for molecular diffusion of protein between the PBS subphase and the monolayer, followed by compression. The π–A isotherm of *E. coli* total lipid and a mixture of colicin E1 and *E. coli* total lipid was collected by injecting colicin E1 (10 μL, 10 mg/mL in PBS) into the subphase (PBS) of the pre-compressed (to 32 mN/m) *E. coli* total lipid (35 μL, 1 mg/mL) monolayer. An equilibration period (20 min) allowed for protein adsorption during which the surface pressure increased and reached a plateau, and was followed by annealing cycles of the mixed monolayer. The ends of the π–A isotherms closest to the x-axis origin for pure colicin E1 and its mixture with *E. coli* total lipid represent the full range of the LB trough barrier.

2.4. Preparation of SLBs by the LB method

Monolayers of *E. coli* total lipid and DOPC were prepared by dropping a lipid solution (45 μL, 1 mg/mL in chloroform) onto the air/buffer interface followed by two annealing cycles and compression to the target pressure (32 mN/m). The first monolayer was deposited by drawing the immersed, freshly cleaved mica sheet up through the air/buffer interface, and the second monolayer was deposited by passing the monolayer that had dried (15 min) on mica down through the interface. The dipping speed (5 mm/min) was the same for all experiments.

2.5. Incubation of SLBs with colicin E1

Two different concentrations (0.001 and 0.1 μM) were prepared for colicin E1 in PBS, and each aliquot (25 μL) was incubated (4 °C) on the surface of an SLB, either *E. coli* total lipid or DOPC, overnight. The residual incubation mixture was gently rinsed from the bilayer with water (3×10 mL), and the SLB dried (4 °C) and then imaged by CM-AFM.

2.6. Atomic force microscopy

The AFM images were collected with an Explorer™ AFM (ThermoMicroscopes, Sunnyvale, CA) equipped with a dry scanner (10 μm in z and 100 μm in x and y) for which the instrument setup has been described elsewhere [17]. Topography and lateral force images were collected simultaneously with contact mode Si_3N_4 probes (ThermoMicroscopes, nominal spring constant = 0.032 N/m, resonant frequency = 17 kHz) with a scan speed of 1 Hz and 500×500 pixel resolution unless otherwise noted. The resolution (nm/pixel) is reported for each image. The same tip, or tips with very similar spring constants were use for all data collection and the set point of the z piezo was always zero during data collection to facilitate image comparison. Lateral force images represent tip–sample interactions for very flat samples such as SLBs, and a convolution of tip–sample interactions with topography for samples with rough surface features. Images were collected under "dry" conditions, processed using horizontal leveling, maximal height adjusted for optimum contrast, and the system background subtracted (SPMLab v. 6.0 software, Veeco).

2.7. Data analysis

The inset of Fig. 2 was calculated (NIMA software) using the molecular weight of colicin E1 and the volume injected (5 μL), assuming that all of the injected protein was adsorbed onto the air/buffer interface. Since the edges of surface features for SLBs are a convolution of the AFM probe and surface feature shapes, heights of features were measured manually using the line measurement

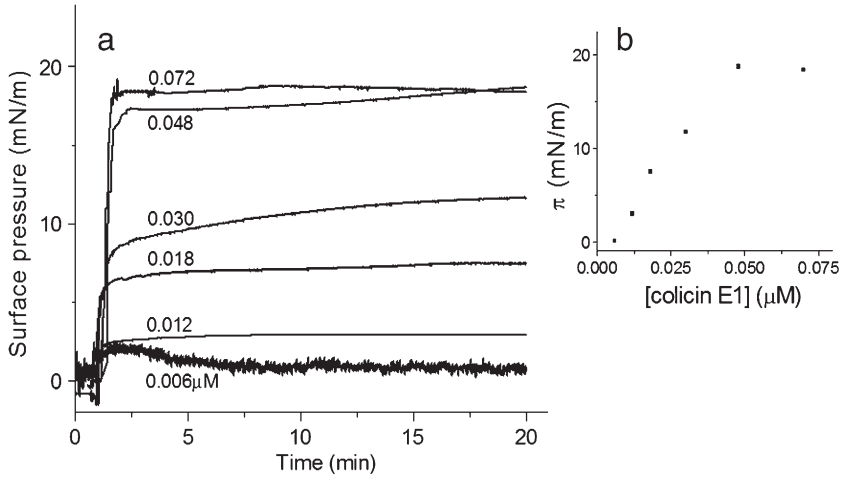

Fig. 1. (a) Surface pressure (π)–time (t) adsorption isotherms for various concentrations (0.006–0.072 μM) of colicin E1 injected into the PBS (pH=7.0) subphase of an LB trough. The concentration of colicin E1 is shown for each curve. The surface pressure of the π–t isotherm recorded at 0.006 μM colicin E1 is scaled up (20×) from the original data. (b) LB surface pressure as a function of colicin E1 concentration following a 20-min incubation period.

option from the middle of one domain to the other, either peak to peak or valley to valley. Heights are expressed as histograms from a large sampling (n>80). Widths of features were also measured manually with the software, and their edges were estimated using the full width at half maximum (FWHM). In some cases, images have been presented enlarged for visual clarity.

3. Results and discussion

3.1. Interaction of colicin E1 with E. coli total lipid at the air/buffer interface

The LB method was used to study the behavior of colicin E1 at the air/buffer interface and the interaction between colicin E1 and a compressed *E. coli* total lipid monolayer. The π–t isotherm of colicin E1 is shown in Fig. 1a. Protein at a series of concentrations was added to the PBS subphase (0.1 M, pH=7.0, 150 mL). The surface pressure increased slightly (to 0.69 mN/m) when protein was added to a final concentration of 0.006 μM in the subphase. There was a steady but nonlinear increase in surface pressure as the protein concentration was increased from 0.012 to 0.072 μM, and the change in surface pressure as a function of protein concentration is shown clearly in Fig. 1b. These results indicate that colicin E1 is amphiphilic, as expected, and that protein injected into the PBS subphase was adsorbed to the air/buffer interface, forming a monolayer. This result is consistent with previous work on the 35 amino acid surfactant protein, SP-C, demonstrating its ability to form a monolayer and amphiphilic character [18,19]. The hyperbolic relationship between surface pressure and protein concentration (Fig. 1b) indicates that the interface is saturated with colicin E1 at a concentration of approximately 0.05 μM. The π–A isotherms of colicin E1 monolayers formed at the air/buffer interface were measured following three annealing cycles. Shown in Fig. 2 are isotherms corresponding to protein concentrations of 0.006 μM (Fig. 2, insert) and 0.012 μM (Fig. 2, dashed line). Extrapolating the tangent from the exponential increase of the isotherm (Fig. 2, insert) gives the area per colicin E1 (1300 Å^2). This value is in good agreement with the molecular dimensions of the C-terminal region of colicin E1 determined by X-ray crystallography [20], ~35 Å×36 Å in its smallest cross-section, corresponding to 1260 Å^2 as compared to its largest cross-section of ~1700 Å^2. The structure of the soluble portion (P190, residues 345–522) of colicin E1 was solved by X-ray crystallography to 2.5 Å [10], providing a model for residues 345–522 including most side chains. This structure describes the soluble portion of the channel forming C-terminal domain and most of the linker region to the R domain. The electrostatic surface potential diagram [21–23] (Fig. 3) of P190 exhibits a positively charged region that spans from one end (Lys 402/403) of the molecule to the other (Lys 346), with contributions from many of the 24 Lys residues (Fig. 3a) and the one Arg residue (209). The dimensions

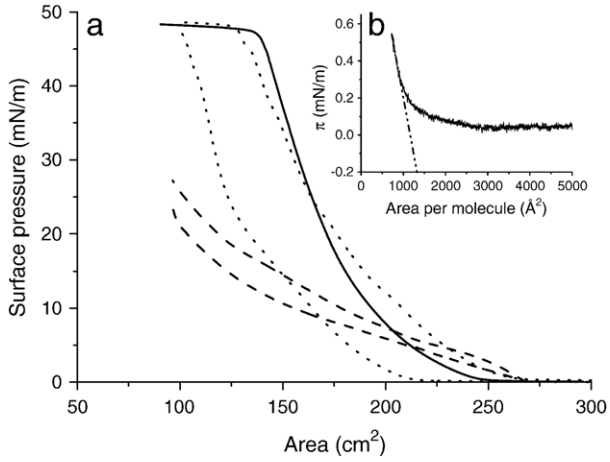

Fig. 2. The π–A isotherms for (——) the compression of an *E. coli* total lipid (35 μL, 1 mg/mL) monolayer on a PBS subphase; (----) compression (upper line) and expansion (lower line) of the colicin E1 monolayer resulting from the addition of colicin E1 to the PBS subphase to a final concentration of 0.012 μM; (·····) compression (upper line) and expansion (lower line) of the monolayer formed by adding colicin E1 (0.012 μM) to the PBS subphase of an *E. coli* total lipid monolayer (35 μL, 1 mg/mL) compressed to 32 mN/m. Insert: compression of the colicin E1 monolayer on PBS buffer by adding protein to the PBS subphase to a final concentration of 0.006 μM, expressed as pressure versus area per molecule.

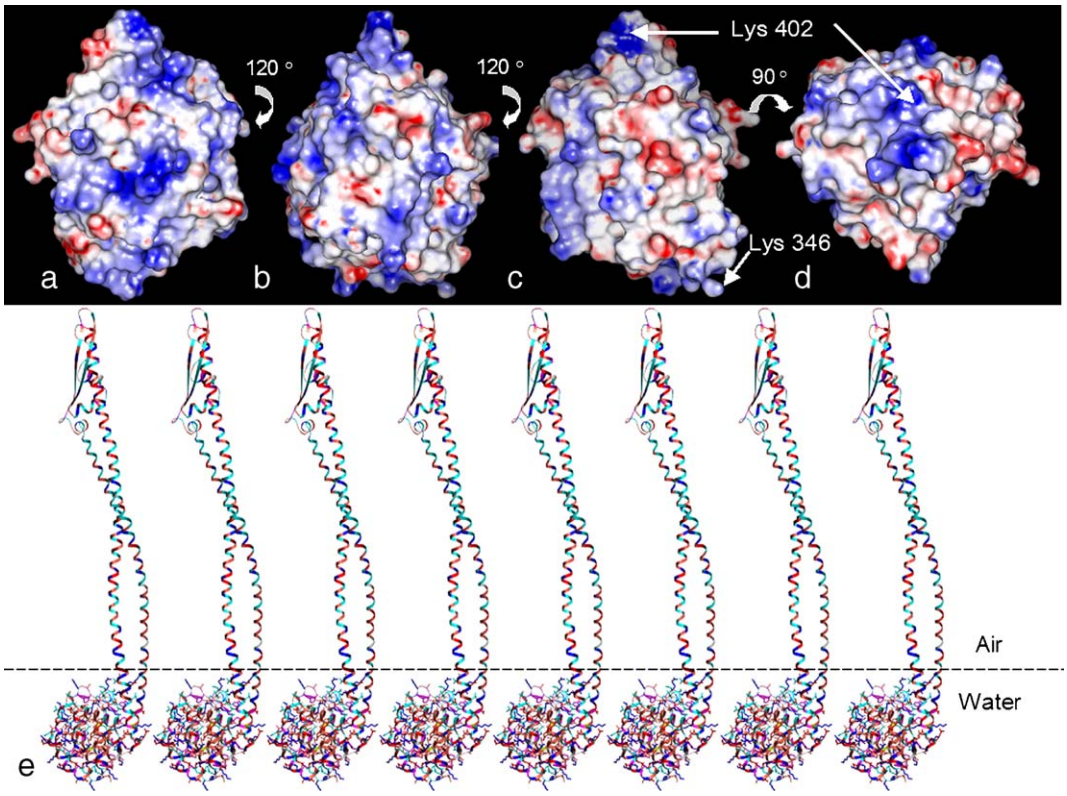

Fig. 3. Electrostatic surface potential diagrams of the soluble portion of colicin E1, colicin E1 P190 [10] calculated using the programs GROMACS [21] and MEAD [22] and rendered by the program PyMol [23]. The surface potential color scheme indicates areas that are neutral (white, 0 kT), positively (blue, + 10 kT) and negatively (red, − 10 kT) charged. The "side view" of the P190 protein has dimensions of approximately 35.5 Å × 47 Å (a) 37.5 Å × 46.3 Å (b) 34.4 Å × 46.4 Å (c) and the "top view" is 35 Å × 36 Å (d). Panels a–c represent a rotation of 120° in the direction shown, while c–d is a 90° rotation in the direction indicated. The positive charges of Colicin E1 P190 form a large surface patch (a) from Lys 346, which represents the N-terminal end of the pore-forming domain to Lys 403 at the other end of the molecule. The proposed monolayer arrangement for colicin E1 at the air–water interface (e) shows repeating subunits of colicin 1a ([35]; pdb file 1CII; residues 204–624) rendered in Setor [36] with standard side chain colors (Lys/Arg = blue). The dashed line represents the air–water interface.

associated with the structures shown in Fig. 3 imply that the C domain would be immersed in water, stacking side by side to form the monolayer, while the T and R domains would extend into the air from the water/air interface (Fig. 3e). The area per molecule of unfolded colicin E1 on the membrane surface has been estimated as 4200 $Å^2$ [8], supporting the idea that although colicin E1 forms a monolayer of folded protein at the air/buffer interface, it requires a charge interaction for unfolding.

The π–A isotherm of *E. coli* total lipid (Fig. 2, solid line) shows the compression of an annealed *E. coli* total lipid monolayer on PBS, slightly different from that at the air/water interface (data not shown) [24]. Although the PBS salts affect the arrangement of lipid molecules at the interface during compression, *E. coli* total lipid is still capable of forming a stable monolayer at the air/PBS interface. *E. coli* total lipid is composed of 57.5% phosphatidylethanolamine (PE), 15.1% phosphatidylglycerol (PG), 9.8% cardiolipin (CL) and 17.6% other lipids, each having hydrocarbon tails of a verified chain length and at least one double bond in the case of unsaturated lipids. The π–A isotherm of *E. coli* total lipid represents a convolution of all isotherms for each individual component as expected. Previous work [24] has shown that an *E. coli* total lipid monolayer compressed to a surface pressure of 32 mN/m, the cell membrane equivalence pressure [25], is predominantly in the liquid condensed (LC) phase [26]. Therefore, we used AFM to investigate the interaction of colicin E1 with *E. coli* total lipid compressed to 32 mN/m at the air/PBS interface, and its interaction with SLBs of *E. coli* total lipid deposited onto mica at surface pressures of 32 mN/m.

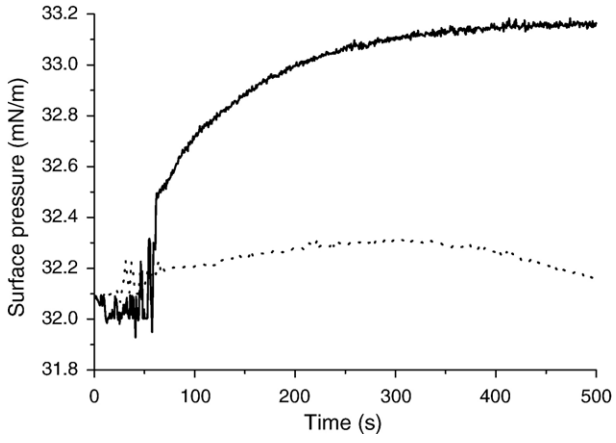

Fig. 4. Surface pressure change as a function of time following the injection of colicin E1 into the PBS subphase of an *E. coli* total lipid monolayer (——) and DOPC monolayer (·····) to a final concentration of 0.012 μM.

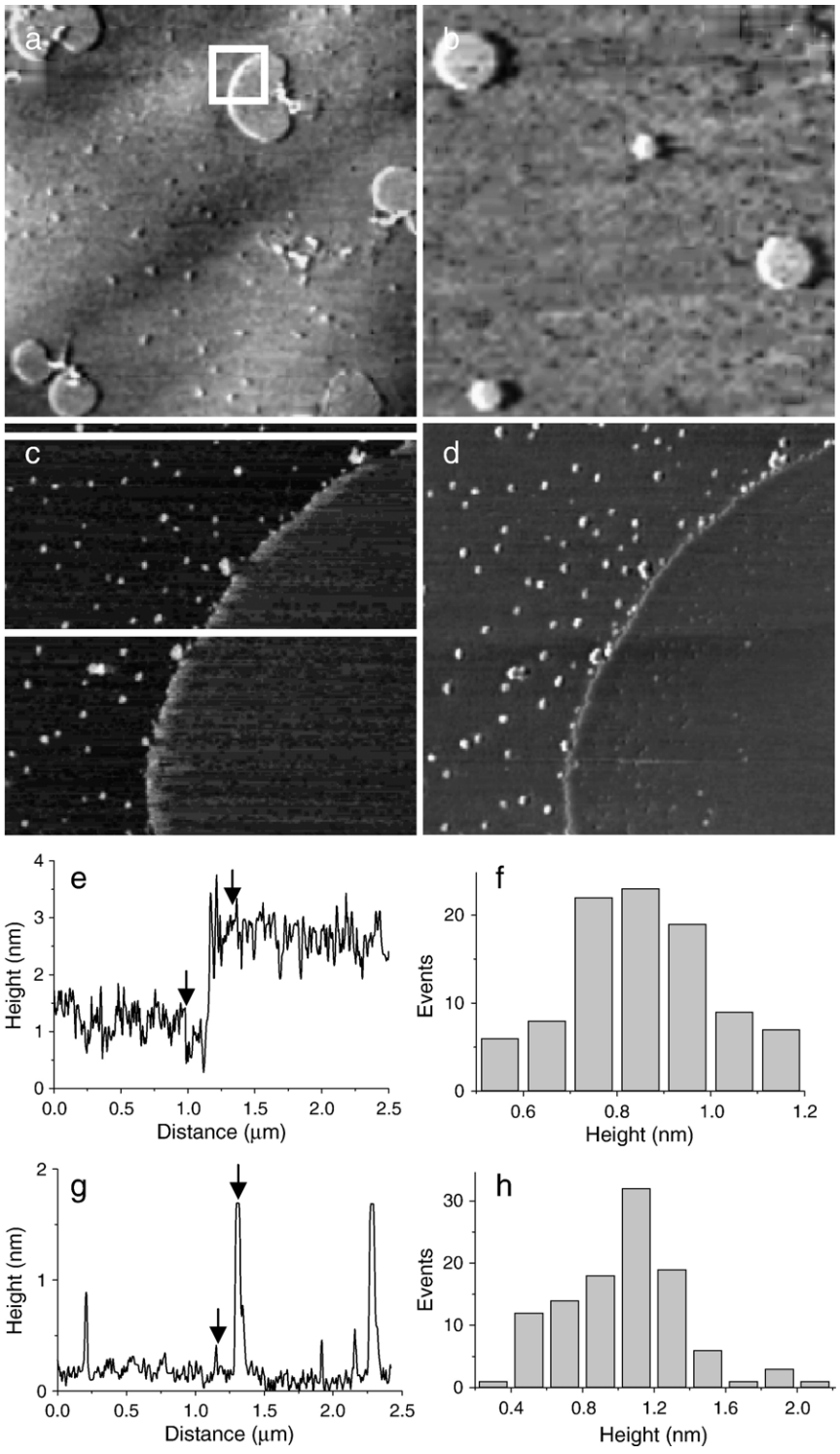

Fig. 5. CM-AFM images (a, b, 20 μm × 20 μm) of *E. coli* total lipid SLBs deposited onto mica at the surface equivalence pressure of *E. coli* (32 mN/m); high resolution (c) topography and (d) lateral force images (20 nm/pixel, scale: 0.07 (black) to 0.79 (white) nA) are shown for the 2.5 μm × 2.5 μm region indicated by the box in image (a). Representative line-scan analyses of the bean-shaped domain and smaller domains shown in (c) are illustrated in (e) and (g) respectively, and their height distributions are shown in (f) and (h), respectively.

Colicin E1 was adsorbed to lipid monolayers, compressed to 32 mN/m, by adding protein (0.012 μM) to the PBS subphase. A zwitterionic lipid, DOPC, was used as a reference lipid for these experiments. For the DOPC monolayer, the surface pressure increased from 32.1 mN/m to 32.3 mN/m (Fig. 4, dotted line) following the addition of protein. DOPC is zwitterionic, and therefore its negative charge is masked as a result of charge neutralization. The 0.6% increase in surface pressure may be the

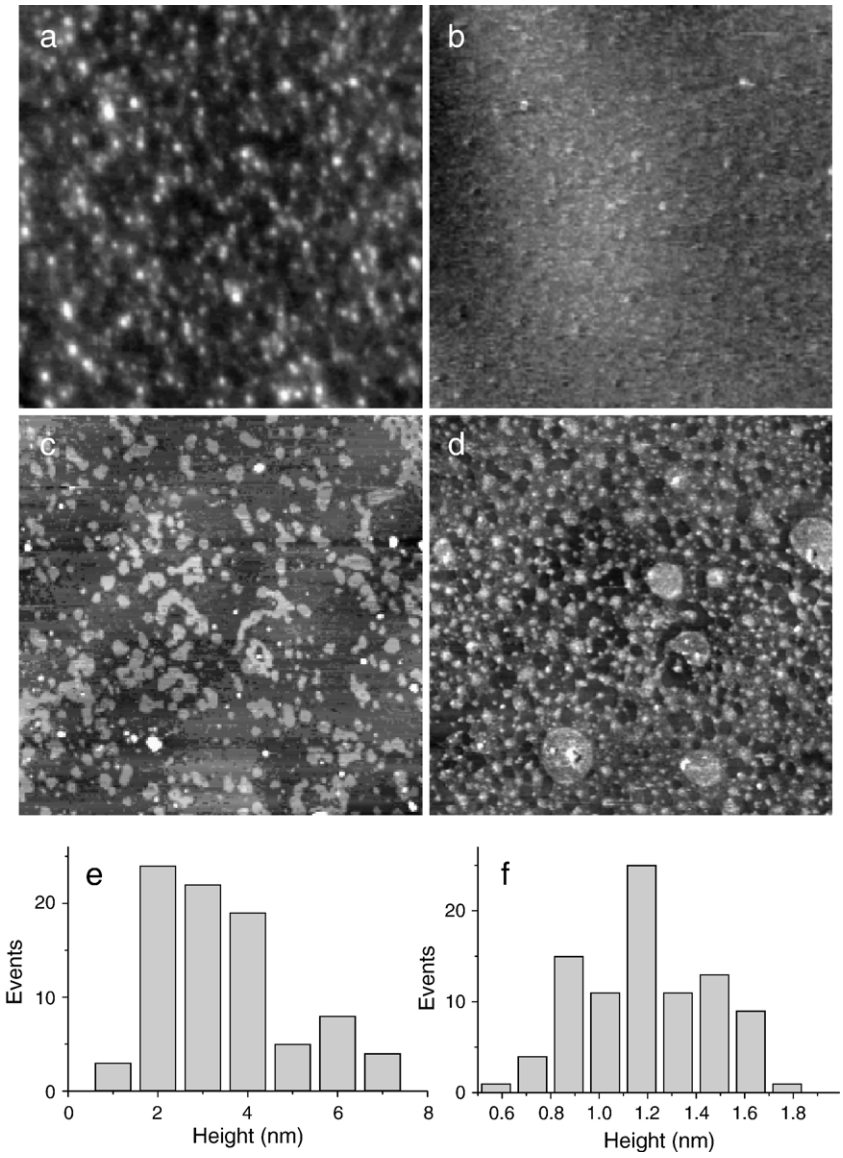

Fig. 6. Topography (left) and corresponding lateral force (right) images (5 μm × 5 μm; 10 nm/pixels) of (a, b) DOPC and (c, d) *E. coli* total lipid SLBs deposited into mica at 32 mN/m and incubated with colicin E1 (25 μL, 0.1 μM) in PBS. The *z*-scale (nA) of the lateral force images ranges from 0 (black) to 1 (white). The height distribution of domains in images (a) and (c) are shown in (e) and (f) respectively.

result of non-specific, or weak electrostatic interactions between the protein (Fig. 3b, c) and lipid. On the contrary, there was a 3.3% increase in surface pressure (32.1 mN/m to 33.2 mN/m) when colicin E1 was added to the subphase of a compressed *E. coli* total lipid monolayer (Fig. 4, solid line). The head group of the PE component of *E. coli* total lipid is zwitterionic [27], while that of PG and CL are negatively charged [28]. The surface potential diagrams of the soluble region of colicin E1 P190 present a large patch with a net positive charge that extends along the "side" of the molecule from "top" to "bottom" (Fig. 3a), and available to interact with the net negatively charged lipid surface to facilitate its insertion. In comparison, there are smaller surface regions that are either zwitterionic or neutral (Fig. 3b, c). There was only a weak interaction between colicin E1 and the zwitterionic DOPC monolayer, providing further support for the idea that the positive charges of colicin E1 associate through electrostatic interactions with the negatively charged head groups (PG and CL) of the *E. coli* total lipid.

The π–A isotherm of *E. coli* total lipid with colicin E1 (Fig. 2, dotted line) was generated by one compression/expansion cycle of the annealed mixed film. The resultant isotherm reveals characteristics of pure protein and *E. coli* total lipid, which indicates that colicin E1 inserts into the *E. coli* total lipid monolayer. It should be noted that the compression and expansion isotherms for both colicin E1 and its mixture with *E. coli* total lipid do not overlap. It is probable that the very large protein molecule is packed in a stable vertical position at the interface with its tertiary structure able to mitigate surface pressure changes during the expansion period, not unlike the hysteresis observed for protein folding and unfolding cycles. At pH 7.0, colicin E1 has been shown to interact with, but not insert into lipid bilayers [6]. The insertion of colicin E1 into the *E. coli* total lipid film is indicative

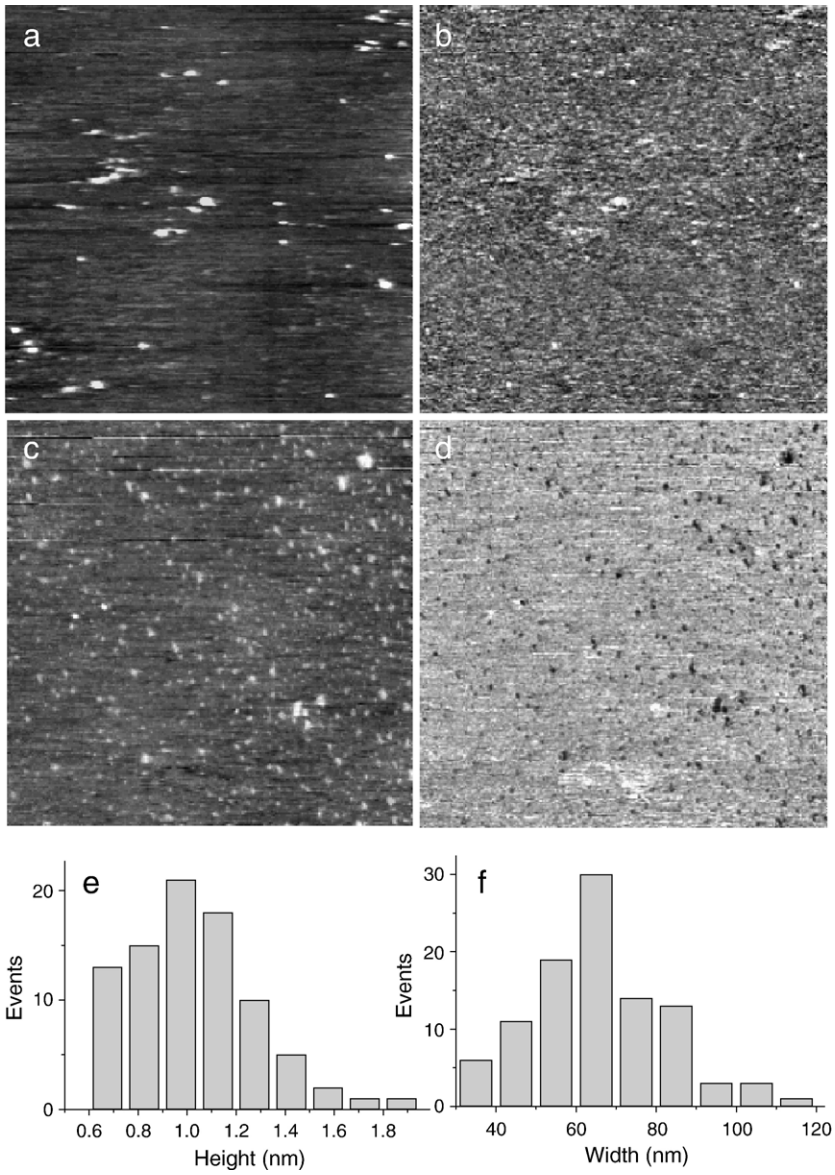

Fig. 7. Topography (left) and corresponding lateral force (right) images (5 μm × 5 μm; 20 nm/pixel) of DOPC (a and b) and *E. coli* total lipid (c and d) SLBs deposited onto mica at 32 mN/m and incubated with colicin E1 (25 μL, 0.001 μM) in PBS. The *z*-scale (nA) of the lateral force images ranges from 0 (black) to 1 (white). The height and width distribution of the domains in image (c) are shown in (e) and (f), respectively. The same tip, or tips with very similar spring constants were used for all data collection and the set point of the *z* piezo was zero.

of the increased deformability of a monolayer versus a bilayer. AFM was used to study the direct interaction of colicin E1 with SLBs that would better mimic a biological system.

3.2. AFM imaging of SLBs

SLBs of *E. coli* total lipid and DOPC were deposited onto mica at the surface pressure of 32 mN/m and imaged by AFM. The DOPC bilayer appears as a uniform film (data not shown) [29–31], while the SLB of *E. coli* total lipid shows bean-shaped or circular domains several microns in size (Fig. 5a, b) and small domains on the nm scale (60 ± 20 nm, Fig. 5c, d). A portion of the *E. coli* total lipid SLB base domain was removed by scanning a small region at high force and subsequent imaging of the surrounding area, indicating a thickness of 3.5 ± 0.2 (data not shown), consistent with bilayers made from phosphatidylcholine (PC) with chain lengths from 13 to 22 carbons. [32,33]. Heights of the bean-shaped and smaller domains are shown in the line scans (Fig. 5e, g) and topography image (white lines in Fig. 5c) as the difference between the taller domains and the surrounding lipid bilayer (shown by black arrows in Fig. 5e, g). The average heights of the bean-shaped and smaller circular domains are 0.85 ± 0.1 nm (Fig. 5f) and 1.1 ± 0.2 nm (Fig. 5h), respectively. Since the lipid monolayer is in its LC phase at this surface pressure, the lipid molecules should be oriented perpendicular to the interface and it is likely that the different height domains reflect lipid chain lengths.

3.3. AFM imaging of lipid SLBs following colicin E1 insertion

SLBs of *E. coli* total lipid and DOPC incubated with colicin E1 (0.1 μM) in PBS (0.1 μM, pH 7.0) were imaged by CM-AFM (Fig. 6). The topography image of DOPC following its incubation with colicin E1 (Fig. 6a) shows dense regions with a height range from 1 to 7 nm (Fig. 6e). The zwitterionic DOPC therefore interacts with colicin E1, likely in a non-specific fashion since the adsorbed protein appears to form large aggregates on the SLB following the long incubation period.

For the SLB of *E. coli* total lipid, the bean-shaped and circular domains disappeared following its incubation with colicin E1, and in their place were irregularly shaped domains (Fig. 6c). The irregularly shaped domains show a distribution of heights, with an average of 1.2±0.3 nm above the bilayer from the histogram shown in Fig 6f, corresponding well with the width of an alpha helix (1 nm) [34]. These results are in agreement with previous work showing that positively charged residues of colicin E1 associate with the negatively charged lipid components, followed by its insertion into the lipid bilayer [8], an idea supported by the electrostatic surface potential diagrams (Fig. 3). The *E. coli* total lipid contains the lipids PG and CL with negatively charged head groups. The lateral force (LF) image (Fig. 6d) shows that a smaller force is required to separate the tip from the sample in regions where there are features 1 nm taller than the surrounding lipid (Fig. 6c), likely resulting from a charge neutralization of the lipid bilayer surface following protein absorption. Although no circular or bean shaped domains were observed in the topography images, some circular domains do appear in the lateral force images, indicating regions with a uniform height but a different surface charge.

At high concentrations of colicin E1 (0.1 μM), there were no visible individual protein molecules, and the protein appears to form large domains. In addition, very large features (1 to 8 nm) that strongly interact with the tip (LF images) were observed, and these are ascribed to large protein aggregates. Therefore, it was necessary to optimize the protein concentration to investigate the effect and localization of individual colicin E1 molecules on the surface of *E. coli* total lipid SLBs. Images of DOPC SLB incubated with lower concentrations of colicin E1 (0.001 μM) reduced protein aggregation on the SLB surface (Fig. 7a and b). For SLBs of *E. coli* total lipid, the topographic features ascribed to the protein show reduced tip–sample interaction (Fig. 7d) indicating charge neutralization of the lipid surface by colicin E1. Corresponding domains visualized in lateral force images of *E. coli* total lipid SLBs, prepared and imaged on different days and with different tips, show identical contrast for tip–sample interaction magnitudes, as do those collected at 0 and 90° (data not shown).

Topography images (Fig. 7c) show the smallest features to be 1.0±0.2 nm high (Fig. 7e) and 70±20 nm wide (Fig. 7f), where the width of features reaches the resolution of the AFM probe (≤50 nm). The height of these features is comparable to the literature value for the diameter of an α-helix (~1 nm) [34], as expected for a protein associated with the surface of a lipid bilayer. The average area (4200 Å^2) estimated per colicin E1 molecule on the membrane surface [8] is also comparable to the value (4900 Å^2) calculated from the AFM measurements. The difference between topography (Fig. 7c) and lateral force (Fig. 7d) images represents tip–sample interactions associated with the various height domains. Lighter regions are indicative of greater tip–sample interactions, while darker regions reflect less tip–sample interaction. These results are consistent with the charge neutralization of the negatively charged head groups of the *E. coli* SLB by colicin E1, and the helical extension and partial immersion of colicin E1 at the membrane interfacial layer as indicated in Cramer's model [8].

4. Conclusion

In conclusion, the amphipathy of colicin E1 was studied by recording the π–t isotherms following its injection into the LB buffer subphase. Colicin E1 formed a monolayer at the air–buffer interface as shown by the π–A compression and expansion isotherm for the LB film. LFM imaging showed that colicin E1 associates with and partially neutralizes the *E. coli* total lipid bilayer, likely through electrostatic interactions, and its insertion into *E. coli* total lipid monolayers at the air/buffer interface was confirmed by surface π–A and π–t isotherms. SLBs of *E. coli* total lipid deposited onto mica at the cell membrane equivalence pressure for *E. coli* imaged by CM-AFM show a base domain 3.5±0.2 nm thick with large bean-shaped domains 0.85±0.1 nm higher than the base domain, and smaller round domains that are 1.1±0.2 nm taller than the base domain. The incubation of colicin E1 on SLBs of DOPC induced protein aggregate formation, indicative of a non-specific interaction, while colicin E1 showed a specific interaction with SLBs of *E. coli* total lipid, its size corresponding to the helical extension and partial immersion model of colicin E1 on the membrane surface. The LB and AFM data, in conjunction with the electrostatic surface potentials of colicin E1 P190, are consistent with an electrostatic interaction between colicin E1 and the head groups of *E. coli* total lipid.

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