Lipopolysaccharide-Dependent Membrane Permeation and Lipid Clustering Caused by Cyclic Lipopeptide Colistin

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ABSTRACT: Polyanionic lipopolysaccharides (LPS) play an important role in regulating the permeability of the outer membrane (OM) of Gram-negative bacteria. Impairment of the LPS-enriched OM is essential in initiating the bactericidal activity of polymyxins. We are interested in how colistin (polymyxin E) affects the membrane permeability of LPS/phospholipid bilayers. Our vesicle leakage experiment showed that colistin binding enhanced bilayer permeability; the maximum increase in the bilayer permeability was positively correlated with the LPS fraction. Addition of magnesium ions abolished the effect of LPS in enhancing bilayer permeabilization. To describe the vesicle leakage behavior from a structural perspective, we performed liquid atomic force microscopy (AFM) measurements on planar lipid bilayers. We found that colistin caused the formation of nano- and macroclusters that protruded from the bilayer by ∼2 nm. Moreover, cluster development was promoted by increasing the fraction of LPS or colistin concentration but inhibited by magnesium ions. To explain our experimental data, we proposed a lipid clustering model where colistin binds to LPS to form large-scale complexes segregated from zwitterionic phospholipids. The discontinuity (and thickness mismatch) at the edge of LPS−colistin clusters will create a passage that allows solutes to permeate through. The proposed model is consistent with all data obtained from our leakage and AFM experiments. Our results of LPS-dependent membrane restructuring provided useful insights into the mechanism that could be used by polymyxins in impairing the permeability barrier of the OM of Gram-negative bacteria.

1. INTRODUCTION
Polymyxins (A−E) belong to a group of cyclic lipopeptides with potent antimicrobial characteristics. Figure 1 shows that the heptapeptide ring of polymyxins is enriched with the unusual cationic diaminobutyric acid (DAB); the C-terminus of the exocyclic tripeptide is fused to the macrocycle at positions 6 and 7. Cationic DABs are located at positions 1, 3, 5, 8, and 9.

Figure 1. Chemical structure of colistin. The amphipathic peptide is composed of a heptapeptide macrocycle, an exocyclic tripeptide, and an acyl tail. Colistin has two hydrophobic domains, including the acyl chain and the hydrophobic patch of the macrocycle at positions 6 and 7. Cationic DABs are located at positions 1, 3, 5, 8, and 9.

Bacterium Bacillus colistinus. Colistin is effective at a few micromolar concentrations to inhibit bacterial growth or kill bacteria. Polymyxins, primarily polymyxin B and colistin, were used as broad-spectrum antibiotics for clinical application starting in the 1950s. Later, clinical experiences showed that polymyxin treatment was often accompanied with neurotoxic and nephrotoxic effects, although the toxicity was reversible upon discontinuation of the drug usage. The usage of polymyxins was gradually abandoned in the early 1980s. Recently, multidrug-resistant (MDR) bacterial pathogens, mostly Gram-negative species such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae, have emerged as a great health threat. Because of their potent antimicrobial activities and low susceptibility to bacterial resistance, polymyxins have received a renewed interest in combating MDR infections. In particular, polymyxins are currently used as last-resort drugs to treat infections (e.g., cystic fibrosis) caused by microbes that are resistant to almost all available antibiotics.

The envelope of Gram-negative bacteria has a complex multilayered architecture, including the outer membrane (OM), the cytoplasmic membrane, and a peptidoglycan layer.
located at the periplasmic space. The OM of Gram-negative bacteria forms a permeability barrier that is essential to bacterial viability. The OM has an asymmetric organization; the outer monolayer is mainly composed of lipopolysaccharides (LPS), whereas the inner leaflet is formed by phospholipids. Compared to phospholipids, polyanionic LPS molecules are better suited for intermolecular interactions to form a supramolecular organization. Structurally, LPS can be divided into three regions: an amphipathic lipid A moiety, a hydrophilic oligosaccharide core (including the inner and outer cores), and an O-antigen chain. It is believed that strong intermolecular interactions of LPS bridged by divalent cations (e.g., Mg\(^{2+}\) and Ca\(^{2+}\)) are the primary mechanism of enhancing the OM stability and limiting the permeation of external agents such as lipophilic antibiotic compounds.\(^8\,9\)

Polymyxins have a high affinity for LPS molecules.\(^10\,−\,13\) This is exemplified by the antendotoxic effect of polymyxins in treating septic shock caused by free LPS molecules (also known as endotoxins).\(^14\) The strong association of polymyxins with LPS also explains the formation of rod-shaped projections from the cell wall of Gram-negative bacteria when exposed to polymyxin B.\(^15\) Although the exact mechanism underlying polymyxin functioning against Gram-negative bacteria remains an open question, impairment of the LPS layer is believed to play a crucial role in initiating the bactericidal activity. Polymyxin binding will cause the displacement of divalent cations that are important for stabilizing the LPS layer by linking phosphates of neighboring LPS molecules. A weakened LPS layer will allow more polymyxins (and other solutes) to traverse the OM.\(^16,\,17\) The mechanism was coined as the self-promoted uptake pathway.\(^16\) Collectively, the first step of polymyxin functioning is to modify the structure and organization of the LPS-enriched OM bilayer. Subsequent bactericidal activity can be carried out by different mechanisms that are still not fully understood. A few of the proposed bactericidal mechanisms include the perforation of the cell wall,\(^18\) formation of molecular contacts between the OM and the cytoplasmic membrane,\(^19\) loss of enzymatic components,\(^20\,−\,22\) and production of reactive oxygen species.\(^22\)

To elucidate the effect of polymyxins on the OM bilayer, many studies have used LPS-containing vesicles or monolayers. Selective examples include the following: (i) electron microscopy showed that colistin disintegrated ribbon-like vesicular structures formed by isolated LPS;\(^23\) (ii) electron spin resonance revealed that polymyxin B had a large impact on the packing property of LPS;\(^24\) (iii) polymyxins inserted into LPS monolayers at the air–water interface as evidenced by an increase in the surface pressure;\(^25,\,26\) (iv) electro-physiological measurements showed that polymyxin B induced electrically active lesions in LPS-containing bilayers;\(^27,\,28\) and (v) nuclear magnetic resonance coupled with computational modeling was used to predict the molecular binding mode of polymyxin B and LPS molecules.\(^29\,−\,31\)

In contrast to the abundant studies of polymyxins interacting with LPS in vesicular or monolayer systems, only a few reports of polymyxin–LPS interactions in a planar bilayer setup have been made. A recent study used LPS/phospholipid mixtures (and deep rough LPS) to prepare oriented multimolecular stacks to mimic the OM bilayer; the authors then used X-ray diffuse scattering to explore the effect of colistin on mechanical properties of the LPS-containing bilayers.\(^32\) Here, we used liquid atomic force microscopy (AFM) to investigate the effect of colistin on nanoscale topographic structures of LPS-containing lipid bilayers. Our AFM-based study is motivated by the facts that (i) AFM is a suitable technique to visually detect structural changes of lipid bilayers induced by membrane-active compounds and (ii) only two AFM-based studies have been reported on polymyxins interacting with LPS; one study was performed on a lipid A monolayer\(^33\) and the other used biotinylated LPS to form a monolayer supported by a densely packed avidin layer.\(^34\)

In this paper, we first used vesicle leakage experiment to study changes in bilayer permeability caused by colistin. The obtained results revealed that colistin-induced bilayer permeation was dependent on the LPS fraction in LPS/phospholipid bilayers. We then used liquid AFM to explore structural changes of planar lipid bilayers. Our AFM measurements showed that the effect of colistin on bilayer topographic structure was dependent on the LPS fraction and the peptide concentration. Importantly, we observed nanoscale clusters that are consistent with the complex formation of colistin and LPS molecules. The segregated lipid clusters could produce membrane lesions (i.e., cluster edges) that are consistent with our vesicle leakage data. We also found that both bilayer permeabilization and cluster development were inhibited by introducing magnesium ions into LPS-containing lipid bilayers. Results from our vesicle leakage and liquid AFM experiments provided useful insights into the mechanism that could be used by polymyxins in impairing the permeability barrier of the OM of Gram-negative bacteria.

2. MATERIALS AND METHODS

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was purchased from NOF America (purity >99%). Lipid stock solutions were prepared by dissolving lipid powders in organic solvents (chloroform and methanol). Calcein disodium salt and lyophilized LPS powder were purchased from Sigma-Aldrich. According to the vendor, LPS was phenol extracted from P. aeruginosa. The source strain is ATCC 27316. Colistin sulfate powder was purchased from Alfa Aesar.

2.1. Vesicle Leakage Experiment. Dry lipid films were prepared in glass test tubes by mixing appropriate ratios of lipid stock solutions. Organic solvents were removed by a gentle stream of nitrogen gas using a 12-position N-EVAP evaporator (Organomation Associates, Inc., Berlin, MA). The samples were further dried by vacuum pumping for ~1 h. The obtained lipid films were hydrated in 30 mM calcein and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.4. For LPS-containing samples, an appropriate amount of an LPS stock solution (in water) was mixed with lipid suspension during the hydration process. We used a Qsonica cup-horn powered by a Fisherbrand model 705 sonic dismembrator to prepare calcein-enclosed unilamellar vesicles. The output power was 50% and the total sonication time was 20 min (1 min on and 30 s off). External calcein was removed by using an AKTA Pure (GE Healthcare) and a gel filtration column (Superdex 200, 10/300GL). The running buffer contained 45 mM NaCl and 10 mM HEPES (pH 7.4).

Colistin sulfate stock solution was prepared in the running buffer (i.e., 45 mM NaCl and 10 mM HEPES pH 7.4). To prepare vesicles treated with different concentrations of colistin, we first mixed the colistin stock solution with the running buffer to achieve different colistin concentrations (final volume of 970 μL). Calcein-enclosed vesicle stock solution (30 μL) was added to the prepared solution with different colistin concentrations. To obtain maximum vesicle
leakage, we substituted the colistin solution with the nonionic surfactant Triton X-100 (final concentration of 20 mM). The vesicle–colistin mixtures were incubated in the dark for 20 h. The incubated samples were transferred to a quartz cuvette. Fluorescence spectrum at 23 °C was obtained using an FP-8300 spectrofluorometer (Jasco Analytical Instruments) and a Peltier thermal cell holder (model EHC-813). The excitation wavelength was 494 ± 2.5 nm. The emission spectrum was measured from 500 to 600 nm (bandwidth of 2.5 nm). Three measurements were performed for each sample. The maximum emission intensity at 515 nm was used for vesicle leakage evaluation. Vesicle leakage % was calculated using the following equation:

\[
\text{Leakage} \% = \left(\frac{I - I_0}{I_{\text{max}} - I_0}\right) \times 100\%
\]

where \(I_0\) is the fluorescence intensity of control vesicles, \(I_{\text{max}}\) is the fluorescence intensity of vesicles treated with Triton X-100, and \(I\) is the fluorescence intensity of vesicles treated with different concentrations of colistin.

2.2. Liquid AFM Experiment. Dry lipid films were prepared by the same procedure as described for the leakage experiment. An LPS stock solution was prepared in ultrapure water. To obtain LPS/POPC mixtures with different weight ratios, an appropriate volume of the LPS stock solution was added to the POPC film. The mixture was repeatedly vortexed and sonicated using a bath sonicator. The lipid suspension was later transferred to a 10 mL glass beaker. Additional ultrapure water was added to the beaker, so the final volume was ~5 mL. Small unilamellar vesicles (SUVs) were prepared using a Branson Ultrasonics Sonifer SFX 250 and a 10 mm flat tip. The total sonication time was 5 min (30 s on and 30 s off). The obtained SUVs were briefly centrifuged before bilayer preparation for AFM imaging.

We used the same procedure for AFM measurements (room temperature) as has been reported elsewhere. Briefly, we injected SUVs into an AFM liquid cell to prepare micr supported planar bilayers. After incubation of ~15 min, we injected ultrapure water to flush out SUVs that did not participate in bilayer formation. AFM images were acquired using a Multimode 8 AFM (Bruker, Santa Barbara, CA). The AFM mode we used is called the PeakForce quantitative nanomechanics (QNM) mode. AFM height images were acquired using a DNP-S10 probe with a scan rate of ~1 Hz and a peak force of ~400 pN. After obtaining several images of the control bilayer, we then injected a colistin solution with a specific concentration (in water). AFM images of colistin-treated bilayers were acquired using the same procedure as for the control bilayer. The obtained AFM images were leveled by subtracting a polynomial background using in-house developed MATLAB scripts.

3. RESULTS

3.1. Vesicle Leakage. We used fluorescence spectroscopy to examine the effect of colistin on bilayer permeability. Lipid vesicles were prepared at several LPS/POPC weight ratios. Note that because the molecular weight of the isolated LPS is unknown, we use weight ratios for LPS/POPC bilayers in this paper. Calcein-enclosed lipid vesicles were prepared by gel filtration. Fluorescence signal from control vesicles is small due to the self-quenching property of the fluorophore (i.e., calcein). When colistin binds to lipid vesicles, the resulting increase in the bilayer permeability will cause an increase in the fluorescence signal. We have previously used this method to evaluate changes in bilayer permeability caused by different compounds. Here, we used vesicle leakage to measure changes in the bilayer permeability after incubating lipid vesicles with colistin for 20 h. The result is summarized in Figure 2. For the LPS-absent POPC vesicles, a maximum permeability increase of ~10% was observed. The addition of LPS at 1:100 yielded a maximum permeability of ~30% (10 μM colistin). A further increase of the LPS/POPC weight ratio to 1:30 and 1:10 resulted in a maximum permeability of ~55% (10 μM colistin). By comparing the maximum leakage % for LPS/POPC bilayers with different LPS fractions, we conclude that colistin-induced bilayer permeation is dependent on the LPS fraction. In addition, because the maximum leakage % is <100% for all LPS/POPC bilayers studied, we conjecture that only a portion of the fluorophore molecules was released from lipid vesicles even when the maximum leakage was achieved. This indicates that colistin did not completely damage lipid vesicles as the surfactant Triton X-100 would. Lastly, we examined the effect of divalent cationic magnesium ions on bilayer permeabilization. This is done by preparing LPS/POPC 1:30 vesicles with 5 mM MgCl₂ both inside and outside. Figure 2 shows that the magnesium ions significantly suppressed the maximum leakage % such that the LPS/POPC 1:30 vesicles suspended in 5 mM MgCl₂ responded similarly to colistin as the POPC vesicles did. Therefore, magnesium ions abrogated the effect of LPS in enhancing bilayer permeabilization caused by colistin.

3.2. AFM Experiment on Planar Lipid Bilayers. We conducted liquid AFM measurements to visually detect nanoscale structural changes of LPS/POPC bilayers induced by colistin. Figure 3 shows the AFM images of an LPS/POPC 1:60 bilayer before and after exposure to 10 μM colistin. The control bilayer exhibited a macroscopically homogeneous organization. The smooth surface structure is also indicated by the height profile along the bilayer surface (Figure 3c). After confirming the quality of the control bilayer, we used a syringe pump to inject a 10 μM colistin solution. Figure 3b shows the modified bilayer structure after exposure to 10 μM colistin. Numerous small clusters with a length scale of ~20 nm were observed. Larger clusters with a length scale up to a few hundred nanometers were also present at several locations. For

![Figure 2](image-url)
simplicity, we will refer to the smaller clusters as “nanoclusters,” and the larger clusters as “macroclusters” or “clusters”. AFM is sensitive to the bilayer normal dimension. The height profile along an arbitrary path showed that the macroclusters have a height of ~2 nm above the background. The height of the nanoclusters is not well-defined by the height profile because the path does not always coincide with the center of the nanoclusters. Nevertheless, the nanoclusters have a height of ~1–2 nm as evidenced by the height profile.

After obtaining the preliminary data of bilayer restructuring, we are interested in the effect of the LPS fraction in regulating colistin-induced lipid clustering. We used AFM imaging to explore bilayer remodeling induced by colistin at two LPS fractions. Figure 4 shows the AFM images of two LPS-containing bilayers (i.e., LPS/POPC of 1:60 and 1:30) before and after treatment with 10 μM colistin. Dispersed clusters with a length scale of a few hundred nanometers were observed for the LPS/POPC 1:60 bilayer (Figure 4b) (note that Figures 4b and 3b are similar except for the image sizes. The nanoclusters in Figure 3b are not distinguishable in Figure 4b.) Increasing the LPS fraction to 1:30 caused a marked increase in the number of the macroclusters (Figure 4d). Despite the striking difference in their numbers, macroclusters in both bilayers had a height of ~2 nm. Collectively, our AFM experiment on two LPS/POPC bilayers with varying LPS fractions showed that the degree of lipid clustering induced by colistin is positively correlated with the fraction of LPS in LPS/POPC bilayers.

Next, we examined the effect of the peptide concentration in mediating colistin–bilayer interactions. We used an LPS/POPC 1:30 bilayer as the control system—AFM image of the control bilayer is the same as Figure 4c. Exposure of the control bilayer to 5 μM colistin caused the formation of many macroclusters (Figure 5a). The height scale is 5 nm. Scale bars are 1 μm.

increased significantly when the peptide concentration was increased to 10 μM (Figure 5b). Note that the cluster height is similar at the two peptide concentrations. Altogether, our AFM measurements of an LPS/POPC bilayer showed that the number of macroclusters induced by colistin is proportional to the peptide concentration.

Lastly, we investigated the effect of magnesium ions on colistin-induced bilayer remodeling. The control bilayer of LPS/POPC 1:30 was prepared from SUVs in the presence of 5 mM MgCl₂. AFM image showed that the control bilayer had a few small spots likely caused by magnesium ion induced LPS aggregation (Figure 6a). Exposing the control bilayer to 5 μM colistin in conjugation with 5 mM MgCl₂ did not result in marked changes in the bilayer structure, although the number of small spots increased moderately (Figure 6b). Notice that the small spots in the presence of 5 mM MgCl₂ had a different
magnesium ions were found to antagonize lipid clustering induced by colistin.

Lastly, magnesium ions were found to antagonize lipid clustering induced by colistin.

We used liquid AFM to determine the impact of colistin on nanoscale structures of LPS-containing lipid bilayers. The AFM experiment revealed that colistin caused a marked change in bilayer topography as evidenced by the formation of nano- and macroclusters. We found that the degree of lipid clustering was dependent on the LPS fraction; more extensive lipid clustering was observed in LPS-containing bilayers. Moreover, no macroclusters were observed in the presence of 5 mM MgCl₂ (Figure 6b). Overall, by comparing Figures 5a and 6b (the same bilayer composition and peptide concentration), we conclude that magnesium ions inhibited the effect of colistin in inducing lipid clustering in LPS-containing bilayers.

4. DISCUSSION

In this study, we used fluorescence spectroscopy and liquid AFM to interrogate the effect of colistin on membrane permeability and nanoscale structures. We are particularly interested in the relationship between the content of the OM-specific LPS and the degree of membrane restructurizing induced by colistin. Our vesicle leakage experiment showed that colistin binding impaired the permeability barrier of LPS-containing lipid vesicles. The maximum leakage % was found to increase with the fraction of LPS. Addition of the divalent cationic magnesium ions inhibited the effect of LPS in enhancing bilayer permeabilization caused by colistin. We used liquid AFM to determine the impact of colistin on nanoscale structures of LPS-containing lipid bilayers. The AFM experiment revealed that colistin caused a marked change in bilayer topography as evidenced by the formation of nano- and macroclusters. We found that the degree of lipid clustering was dependent on the LPS fraction; more extensive lipid clustering was observed in LPS-containing bilayers at a larger fraction of LPS. In addition, more clusters were formed at higher peptide concentrations. Lastly, magnesium ions were found to antagonize lipid clustering induced by colistin.

We used a mixture of LPS and phospholipids to prepare vesicular and planar lipid bilayers. Our choice of the lipid composition was based on several considerations. First, it has been shown that LPS/phospholipid bilayers are suitable for evaluating antimicrobial peptide−LPS interactions. Secondly, there is no experimental evidence to definitively prove that the outer monolayer of the OM is exclusively composed of LPS. Third, the antibacterial activity of polymyxins is correlated with the content (and the chemical structure) of LPS. Complete loss of LPS was reported to serve as a mechanism for developing polymyxin resistance by A. baumannii. Similarly, LPS in cell wash solution was found to reduce the susceptibility of P. aeruginosa to colistin. Collectively, studies of colistin interacting with LPS/phospholipid bilayers are useful to reveal the mechanism of OM permeation caused by polymyxins.

Owing to their opposite charge states, colistin and LPS can form stoichiometric complexes. The strong binding affinities of polymyxins to LPS have been broadly demonstrated by using fluorophore-tagged polymyxins. There are two driving forces directing the recognition of polymyxins toward LPS. The initial binding is facilitated by long-range electrostatic interactions between the negatively charged phosphates of LPS and the positively charged DAB of polymyxins. After initial binding, hydrophobic interactions between the hydrophobic tails of the lipid A motif of LPS and the hydrophobic domains of polymyxins can further increase the binding affinity of the peptides. Many efforts have been made to elucidate the complex structure of polymyxins binding to LPS at a molecular level. A comprehensive summarization can be found in the review work by Velkov and co-workers.

In essence, the polycationic heptapeptide ring of polymyxins resides in the vicinity of the interface between the lipid A motif and the oligosaccharide core, whereas the acyl tail of polymyxins penetrates into the hydrophobic core of LPS. The proposed complex models are in line with the electrostatic and hydrophobic driving forces in stabilizing the binding of polymyxins to LPS. Unlike polyamionic LPS, zwitterionic phospholipids have a weak association with polymyxins.

The distinctive binding affinities of colistin to LPS and POPC are likely responsible for the nano- and macroclusters observed in our AFM experiment. Our observation of lipid clustering has some similarity to an earlier study, which used a monolayer formed by Escherichia coli total extract (no LPS). Although their AFM experiment was performed in air, the authors obtained circular protrusions (height of ∼1 nm) with a radius of 20 or 120 nm, depending on the concentration of polymyxin B. Another study using fluorescence polarization also reported domain formation of polymyxin B and phosphatidic acid in the presence of phosphatidylcholine. To explain the clusters observed in our AFM experiments, we propose a schematic model of colistin bound to an LPS/POPC bilayer (Figure 7). The insertion of colistin causes lipid redistribution such that LPS and colistin segregate away from POPC to form isolated clusters. The cluster and the POPC patch have a height difference of ∼2 nm because of the bulky macrocyclic ring of colistin (and the ordered oligosaccharide core of LPS). The probability of cluster formation is larger at higher LPS contents (and colistin concentrations). Therefore, the proposed model is in line with our observation of lipid clustering as a function of the LPS fraction (and the peptide concentration). Magnesium ions have a strong binding affinity to LPS. Introduction of magnesium ions will competitively inhibit the binding of colistin to LPS. Consequently,
magnesium ions will reduce the development of LPS–colistin clusters (Figure 6).

Our vesicle leakage experiment showed that colistin increased the permeability of LPS/POPC bilayers; the maximum leakage % increased with the LPS fraction. Membrane perforation is a popular mechanism proposed for the bactericidal activity of polymyxins. LPS tends to decrease bilayer permeability by having a tighter packing of acyl chains.78 Permeability enhancement of LPS-containing bilayers (including the OM) can be achieved by pore formation or an alteration in lipid packing.16,42,56 On the basis of electro-physiological measurements, Seydel and co-workers proposed pore-like lesions induced by polymyxins in asymmetric LPS bilayers.27,28 Our high-resolution AFM imaging did not show transmembrane pores induced by colistin. Note that we have previously observed <10 nm-sized pores using the same AFM setup.57 Instead of transmembrane pores, we argue that the hypothetical lesions could correspond to cluster edges proposed in our lipid clustering model. Owing to the discontinuity (and the height mismatch) at the edge of LPS–colistin clusters, solutes will have a larger probability of permeating through the cluster edge. The total length of the cluster perimeter increases with the LPS fraction. Therefore, our lipid clustering model can account for the leakage behavior as a function of the LPS fraction in LPS/POPC vesicles. Because magnesium ions inhibited cluster development (Figure 6), the probability of solutes permeating through cluster edges is significantly reduced. Therefore, our lipid clustering model also supports the role of magnesium ions in abrogating colistin-induced bilayer permeabilization.

5. CONCLUSIONS

Here, we report the effect of cyclic lipopeptide colistin on membrane permeability and nanoscale structures of LPS-containing lipid bilayers. Fluorescence spectroscopy based vesicle leakage experiment revealed that colistin binding impaired bilayer integrity, leading to an enhancement of the bilayer permeability. The maximum increase in the bilayer permeability was positively correlated with the LPS fraction in LPS/phospholipid bilayers. Addition of the divalent cationic magnesium ions suppressed the effect of LPS in enhancing bilayer permeabilization. We used liquid AFM to gain a structural perspective of colistin-induced membrane remodeling. We found that colistin caused the formation of nano- and macroclusters that protruded from the bilayer by ~2 nm. Moreover, cluster development was enhanced by increasing the fraction of LPS or colistin concentration but abolished by magnesium ions. To explain our experimental data, we propose a lipid clustering model where LPS and colistin form large-scale clusters that are segregated from the POPC patch. The discontinuity (and the thickness mismatch) at the cluster edge will create a passage that allows solutes to permeate through. The degree of bilayer permeation is proportional to the total length of the cluster perimeter. Because more clusters are formed at higher LPS contents, our lipid clustering model supports the vesicle leakage data.

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Notes
The authors declare no competing financial interest.

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