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Review

DAPTOMYCIN, its membrane-active mechanism vs. that of other antimicrobial peptides

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ABSTRACT

Over 3000 membrane-active antimicrobial peptides (AMPs) have been discovered, but only three of them have been approved by the U.S. Food and Drug Administration (FDA) for therapeutic applications, i.e., gramicidin, daptomycin and colistin. Of the three approved AMPs, daptomycin is a last-line-of-defense antibiotic for treating Gram-positive infections. However its use has already created bacterial resistance. To search for its substitutes that might counter the resistance, we need to understand its molecular mechanism. The mode of action of daptomycin appears to be causing bacterial membrane depolarization through ion leakage. Daptomycin forms a unique complex with calcium ions and phosphatidylglycerol molecules in membrane at a specific stoichiometric ratio: $Dap_2Ca_3PG_2$. How does this complex promote ion conduction across the membrane? We hope that biophysics of peptide-membrane interaction can answer this question. This review summarizes the biophysical works that have been done on membrane-active AMPs to understand their mechanisms of action, including gramicidin, daptomycin, and underdeveloped pore-forming AMPs. The analysis suggests that daptomycin forms transient ionophores in the target membranes. We discuss questions that remain to be answered.

1. Introduction

Daptomycin is one of a few membrane-active antimicrobial peptides (AMPs) that have been approved by the U.S. Food and Drug Administration (FDA) for clinical use [1,2]. Following increased clinical usage, bacterial pathogens began to develop resistance to daptomycin [3]. The recent transition of daptomycin to generic status is projected to dramatically increase availability, use, and clinical failure [4]. Many daptomycin analogues are being developed, aiming to improve its potency or to counter the bacterial resistance [5–9]. However, there is no guiding principle for developing the daptomycin substitutes, because the molecular mechanism of how daptomycin permeabilizes bacterial membranes has not been clarified. What happens when molecules of daptomycin bind to a lipid domain? We believe that the answer is within the domain of biophysics governing peptide-membrane interactions. This review will provide a summary of what we know about the molecular mechanisms of membrane-active AMPs and suggest further research on daptomycin.

AMPs for therapeutic applications were recently reviewed by Chen and Lu [2]. Among the 3156 AMPs listed in the Antimicrobial Peptide Database (APD) [10], which includes peptides and lipopeptides, only three have been approved by FDA. (Chen and Lu also included four lipoglycopeptide antibiotics, i.e., vancomycin and three derivatives, in their AMP review.) The three approved AMPs are gramicidin, daptomycin, and colistin. All were discovered in or derived from Gram-positive bacteria found in the soil. In contrast, the great majority of AMPs listed in the APD are the components of innate immunity found in varieties of animals. So far none of them have been approved for therapeutic applications [11].

The most important distinction between the AMPs and the conventional antibiotics is that the former target the lipid domains of the cell membranes whereas the latter target specific receptors that include proteins, DNA, RNA, ribosomes or specific lipids such as lipid II [12]. Compared with receptor-specific antibiotics, the mechanisms of membrane-active AMPs are much less clear; that is, what happens after the AMPs reach the lipid domains is often ambiguous. Generally speaking the actions of conventional antibiotics disrupt the bacterial metabolism that limits the growth of bacteria; i.e., antibiotics are bacteriostats. In contrast, AMPs are bacteriocidal, often described as causing membrane lysis. But in fact, at the concentrations comparable to minimum inhibition concentrations (MICs), AMPs do not cause lysis of lipid membranes (for reasons see below). It has been pointed out [13,14] that cell membrane lysis is likely a secondary effect due to activation of autodigestive enzymes. At least, this appears to be the case for daptomycin as will be described below. Direct studies of AMPs with live bacteria [15–19] are also complicated by the presence of an outer membrane or

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a cell wall. As a result, dispute on the membrane-active AMP mechanisms has been common.

To find ways to clarify the membrane-active mechanisms of AMPs, we have studied the action of AMPs on live bacteria spheroplasts and directly compared with their action on giant unilamellar vesicles (GUVs) similar in size [20–22]. A spheroplast is a bacterium (for example, *E. coli*) with its outer membrane removed; as a result, its shape is spherical, comparable to a giant lipid vesicle. (These spheroplasts could revert back to normal form of *E. coli* when returned to a growth medium [20].) First, we studied the physical properties of spheroplast membranes in comparison with GUVs [20]. We then used this knowledge to investigate the phenomena of pore-forming AMPs directly attacking the cytoplasmic membranes of *E. coli* spheroplasts. We developed a procedure of fluorescence recovery after photobleaching (FRAP) to examine the dye leakage through the bacterial membranes as the AMPs in solution attacked the membranes. The permeability through the bacterial membrane increased in a sigmoidal fashion as the AMP binding increased in time, exhibiting a cooperative behavior of AMPs. The analysis of FRAP showed that the fluxes of dye molecules into and out of the cell were consistent with diffusion of molecules through a number of pores that increased with binding of AMPs and then saturated to a steady level. The study confirms that the pore forming activities by LL37, melittin and alamethicin observed in GUVs are reproduced in spheroplasts. Indeed the steady-state membrane permeability induced by these three pore-forming AMPs is quantitatively the same in spheroplasts as in GUVs.

However, this pattern of pore formation was not observed with daptomycin or with the metabolic inhibitor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) [23–25]. CCCP and daptomycin are each known to cause ion leakage. The ion leakage by CCCP and daptomycin would lead to a dye leakage pattern in *E. coli* spheroplast different from the pore-formation pattern. Also both did not cause any dye leakage through the membrane of a GUV. There are however interesting dissimilarities in details that reveal differences between bacterial and lipid membranes. Spheroplast membranes were permeabilized by a wide range of AMP concentrations to the same steady-state membrane permeability. In contrast, only a narrow range of AMP concentrations permeabilized GUVs to a steady state level. Tension in GUVs also influences the action of AMPs, whereas the spheroplast membranes are tensionless. With this understanding, our results provide a strong support for using model membranes to study the action of AMPs on bacterial membranes [21,22].

In the following we review the known molecular mechanisms of membrane-active AMPs. Because the literature often cites all possible effects by AMPs on lipids as possible mechanisms, we want to stress that our discussion is limited to the action of AMPs at their therapeutic concentrations between ~0.1 to ~100 micromolar. At the nanomolar range, AMPs often exhibit transient events of ion conduction across a membrane under an applied electric potential [26–28]. At concentrations higher than 100 micromolar, these molecules could act like surfactants or detergents that cause lysis or disintegration of lipid bilayers, similar to effect of surfactants at a concentration exceeding their critical micellar concentrations [29,30]. These effects observed at very low or very high AMP concentrations are irrelevant to our discussion here.

2. Gramicidins

The gramicidins are linear 15-amino acid polypeptides produced by *Bacillus brevis*. The nature product called gramicidin D is a mixture of gramicidin A (80%), B (5%), and C (15%). The dominant component gramicidin A differs from B and C in the position 11. The primary structure of a gramicidin has alternating D and L amino acids, preponderantly hydrophobic and has no ionizable side chains. The substance is very insolvable in water and adsorbs very strongly at lipid membranes. Gramicidin D was approved by the FDA in 1955 as a constituent in Neosporin® for control of Gram-positive infections [31].

In the presence of a minute amount of gramicidin, in many types of lipid bilayers, the membrane current under fixed applied potential fluctuates in a single-channel step-like manner [32]. All three gramicidins have closely similar ion conducting properties [33]. The ion conduction mediated by gramicidin in bilayer membranes occurs due to the formation of a cylindrical transmembrane channel by two monomers, each a single-stranded $\beta^{6.3}$ helix, linked head-to-head (head = formyl end) by six hydrogen bonds at their N termini [34,35]. The gramicidin channel conducts monovalent cations only. The monovalent cation binding sites are inside the channel at 9.6 $\AA$ from the channel midpoint as determined by X-ray diffraction [36]. Divalent cations bind at the mouth of the channel on each end, 13$\AA$ from the channel midpoint, that block the ion conduction [36].

To determine the dynamics of gramicidin channel formation in membranes, we used a modified gramicidin with its formyl group replaced by a *tert*-butoxycarbonyl (BOC) group, such that the channel is 5-order-of-magnitude destabilized relative to the native channel [37]. X-ray in-plane diffraction found that the signal was dominated by gramicidin in monomeric β helix form [37]. This showed that in membranes, gramicidin exists as both dimers and monomers in kinetic equilibrium. Interestingly the equilibrium constant of the monomer-dimer kinetics depends on the membrane thickness, with the thicker membranes favoring the monomers [38,39]. This was explained by assuming that the membrane thickness is locally hydrophobic-matching the dimeric channel length (Fig. 1). This local membrane deformation due to the hydrophobic-matching incurs an increase in free energy that affects the dimeric channel's lifetime [39]. That in turn explains the membrane-thickness dependence of the gramicidin ion conductivity [38,39]. The clarification of the molecular mechanism of gramicidin demonstrates that ion conduction is indeed an antibiotic mechanism. We know that the presence of cholesterol in mammalian cell membranes has a membrane-thickening effect [40]. This could be a protective factor for the animal cells against the attack of gramicidin.

3. Pore-forming AMPs

Examples include alamethicin, magainin, LL37, melittin, protegrin and many others. They are by far the most extensively studied AMPs [41,42]. However, therapeutic applications of magainin and protegrin derivatives have failed to win the FDA approval so far [11]. In terms of molecular mechanism, the easiest to understand are the linear peptides such as alamethicin, melittin, magainin or LL37. They form amphipathic helices with a high affinity for binding to the membrane surface. Indeed oriented circular dichroism (OCD) [43] showed that helical axes are parallel to the plane of the membrane [44–46]. Such a surface-adsorbed peptide occupies space in the headgroup region of the phospholipid molecules (i.e., the interface) in the bilayer, but does not extend all the way to the center of the bilayer. In order that there not be any empty space underneath the peptide, the lipid chains must be distorted from a smooth planar bilayer to fill the space [47]. As a result, the membrane thickness decreases in proportion to the peptide to lipid molar ratio P/L [48–50].

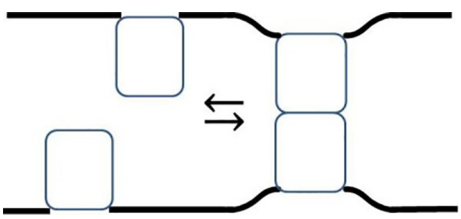

Fig. 1. Gramicidin monomers (the boxes) are embedded in lipid monolayers which can dimerize to form ion conducting channels. The membrane locally deforms to match the channel's length. The resulted membrane deformation incurs a deformation free energy that influences the channel life-time which in turn determines the ion conductivity.

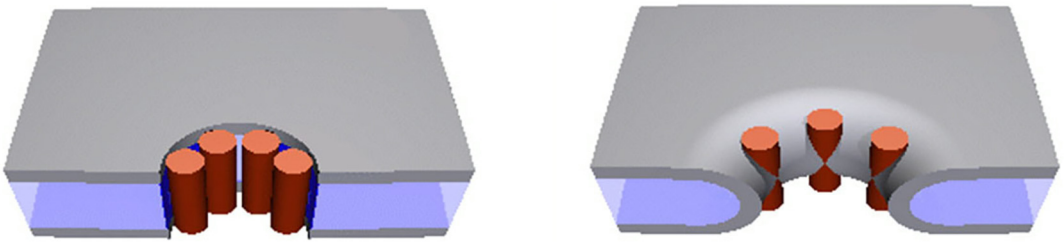

Fig. 2. Peptides adsorbed on the membrane surface are not shown; their helical axes are in the plane and the density is P/L*. The peptides in excess of P/L* are perpendicular to the membrane (red cylinders) forming either the barrel-stave pores (left) or toroidal pores (right).
(The figures are reproduced from ref. [60].)

Importantly the distribution of the peptides on the bilayer surface is spread out without aggregation, as carefully measured by fluorescence energy transfer experiments (FRET) [51–54]. This finding is in agreement with a theoretical argument that the interfacial-bound peptides experience mutually repulsive membrane-mediated interaction [55]. Furthermore, the AMPs in such a surface binding state, do not cause any molecular leakage across the membrane [56].

Since the peptide participation increases the interfacial area and correspondingly thins the membrane (due to the conservation of the total chain volume), there is a limit as to how many peptides can participate per unit area of the membrane, or equivalently there is a critical limit to P/L, called P/L*. When P/L exceeds P/L*, the excessive peptide helices change orientation to become perpendicular to the plane of the membrane, as shown by OCD [44–46].

We found that there are two ways for a membrane to accommodate the perpendicularly oriented peptide helices. In one way a number of peptide helices assemble in a barrel-stave fashion around a pore (Fig. 2), hence called the barrel-stave model, first envisioned by Baumann and Mueller in 1974 [57]. In this way the peptides exceeding P/L* are no longer in the interface, the membrane thickness remains constant for P/L exceeding P/L* [50]. We found that alamethicin forms barrel-stave pores [58].

Another way for a membrane to accommodate the excessive peptides beyond P/L* is to form toroidal pores [59,60]. Imagine a wormhole through a lipid bilayer—in which the lipid monolayer bends and stretches through the hole such that the hole is lined by the lipid headgroups (Fig. 2) [59,60]. Pores are created to increase the interfacial area without increasing the planar membrane area, so as to accommodate the peptides exceeding P/L*. All peptides remain in the interface, but the peptides exceeding P/L* are distributed in the interface of the pore lumen. This is exactly what we found for melittin and similar peptides by X-ray diffraction [61,62]. Almost all the pore-forming AMPs, with the exception of alamethicin, form toroidal pores because they are positively charged, not compatible with the barrel-stave model due to electrostatic repulsion [60].

In short, pore formation is how the membrane accommodates the bound peptides in excess of P/L*. The structures of both pore models have been confirmed by the electron density distribution obtained by multiwavelength anomalous X-ray diffraction [58,61]. Note that pore-forming AMPs always form a massive number of pores, but only when their concentrations in membrane exceed a critical value P/L* [62].

4. Daptomycin

The molecular structure of daptomycin (reproduced in Fig. 3) is crucial for understanding its mechanism. For instance, this molecular structure is not compatible with channel formation like gramicidin, nor is it compatible with pore formation like alamethicin or melittin. Equally important is the fact that its antibacterial activity is calcium ion-dependent [63] and correlates with the target membrane's content of phosphatidylglycerol (PG) [64]. The most consistent report on the action of daptomycin is causing bacterial membrane depolarization [65–68] without lysis of bacterial membranes or the creation of large pores [69,70]. Daptomycin was approved in 2003 by the FDA to treat or prevent infection with Gram-positive pathogens [71].

Early reports of daptomycin aggregations in solution [73] led to speculations that aggregation in solution is a precondition for daptomycin's antibacterial activity [74,75]. However X-ray small angle scattering [72] showed that daptomycin below 0.5 *mM* in a solution of the physiological Ca^{2+} concentration (~1 mM) is monomeric. Therefore at the therapeutic concentration (< 50 μM) daptomycin is monomeric in solution. Accordingly, all experimental results reported below used Ca^{2+} concentrations $\lesssim$1 mM. At high Ca^{2+} concentrations, daptomycin aggregates even in the absence of PG [72]. Fig. 4 shows the CD spectra of daptomycin with Ca^{2+} and DOPC/DOPG vesicles (see also [76]). Spectrum A is the daptomycin monomeric spectrum, obtained in the absence of either Ca^{2+} or PG. Spectrum B is obtained in the presence of excessive Ca^{2+} and PG. In the intermediate ranges of Ca^{2+} and PG concentrations, the spectra are linear combinations of A and B, indicating that daptomycin exists only in either A state or B state. No other CD spectra were detected. The detailed stoichiometric measurement determined that in the B state the daptomycin:Ca^{2+}:PG ratios are 2:3:2 [72].

Daptomycin contains two side chains with chromophores in Trp1 and Kyn13, each has a strong absorption band near 225 nm (see Fig. 4a). The spectra A and B suggest an exciton coupling between two chromophores that gives rise to a splitting of the excited state into two components of opposite signs at higher and lower wavelength of 225 nm, one of which arises from an in-phase combination of the two monomeric excitations and the other from the out-of-phase combination [77–79]. Since spectrum A is of monomeric daptomycin, the coupling is most likely intramolecular between Trp1 and Kyn13. We looked for such evidence from NMR, but NMR studies [76,80–83] were typically performed at high peptide concentrations (> 1 mM). Such samples are prone to aggregation, particularly in the presence of Ca^{2+} [82]. Nevertheless, in Ca^{2+}-free samples, Rotondi and Gierasch [82] detected interaction between the aromatic side chains of Trp1 and Kyn13 by short-range nuclear Overhauser effect.

The flip of the sign between spectrum A and B suggests that the B state exciton coupling is no longer intramolecular. Since the stoichiometric ratios of the B state are 2:3:2 for daptomycin/Ca^{2+}/PG, we suggest that the B spectrum is due to the dimeric complex $Dap_2Ca_3PG_2$. The intermolecular coupling within this dimeric complex gives rise to the exciton coupling in spectrum B.

Very importantly, we found that when the DOPC/DOPG vesicles were replaced by L-glycerol 3-phosphate (i.e., the headgroup of PG without the fatty acid chains), the CD showed only the A spectrum [72]. Daptomycin in solutions containing Ca^{2+} and PG headgroups without lipid chains is in the A state. This tells us that the B state requires the PG lipids oriented in a membrane. There is clear evidence of daptomycin oligomerization (or clustering) in membrane provided by fluorescence resonance energy transfer (FRET) [84–86]. The FRET signal is consistent with either the formation of dimeric complex $Dap_2Ca_3PG_2$ or larger oligomerizations $(Dap_2Ca_3PG_2)_n$ with $n > 1$.

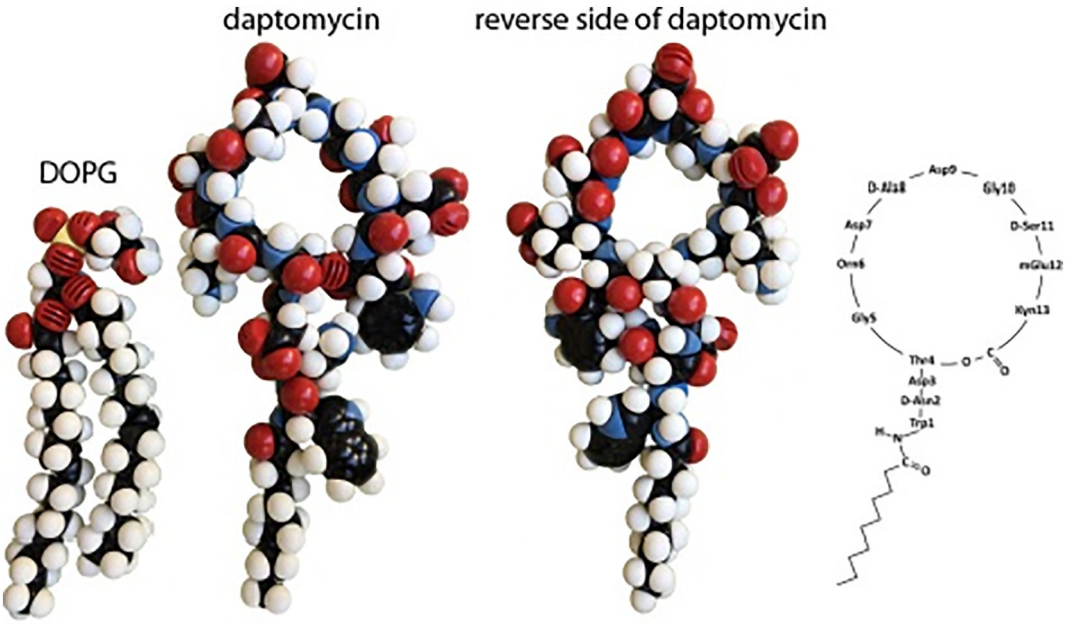

Fig. 3. Structure formula of daptomycin.
Reproduced from ref. [72].

Daptomycin causes transmembrane ion conduction but no transmembrane molecular leakage [69,70]. There are two well-known molecular devices that conduct ions across a membrane, i.e., ion channels and ionophores. Despite numerous suggestions that daptomycin forms ion channels in bacterial membranes [65,66,73,87], the ion conduction induced by daptomycin is not consistent with ion channels. 1) Müller et al. [68], who studied the effect of daptomycin on *B. subtilis*, argued against the existence of daptomycin ion channels based on their findings that the effect of daptomycin is extremely weak compared with the effect of standard ion channels or ionophores such as gramicidin or valinomycin. 2) Direct comparison of daptomycin with ionomycin [88] found that the ion leakage effect of daptomycin is three orders of magnitude smaller than that of ionomycin, consistent with the finding of Müller et al. that daptomycin is a weak ion leaker. 3) Daptomycin induces only transient ion leakage. In experiments using large unilamellar vesicles [88], daptomycin causes ion leakage only when the molecules are introduced into the vesicle suspension for the first time. Afterwards they cease to induce ion leakage. 4) In experiments observing potassium ion leak-out from lipid vesicles [88], we compared daptomycin with gramicidin and valinomycin. We found that gramicidin-channels and valinomycin-ionophores are transferrable between lipid vesicles but not daptomycin's ion-conduction complexes. All of these are inconsistent with the hypothesis that daptomycin forms ion channel. Figs. 5 and 6 [88] further show the direct comparison of daptomycin with two well known ionophores, ionomycin [89] and CCCP [23–25]. Based on these observations, we suggest that daptomycin forms transient ionophores.

Recently, Seydlova et al. [91] claimed to have shown for the first time that daptomycin caused molecular (propidium iodide) leakage through *B. subtilis* membranes, which they interpreted as due to discrete

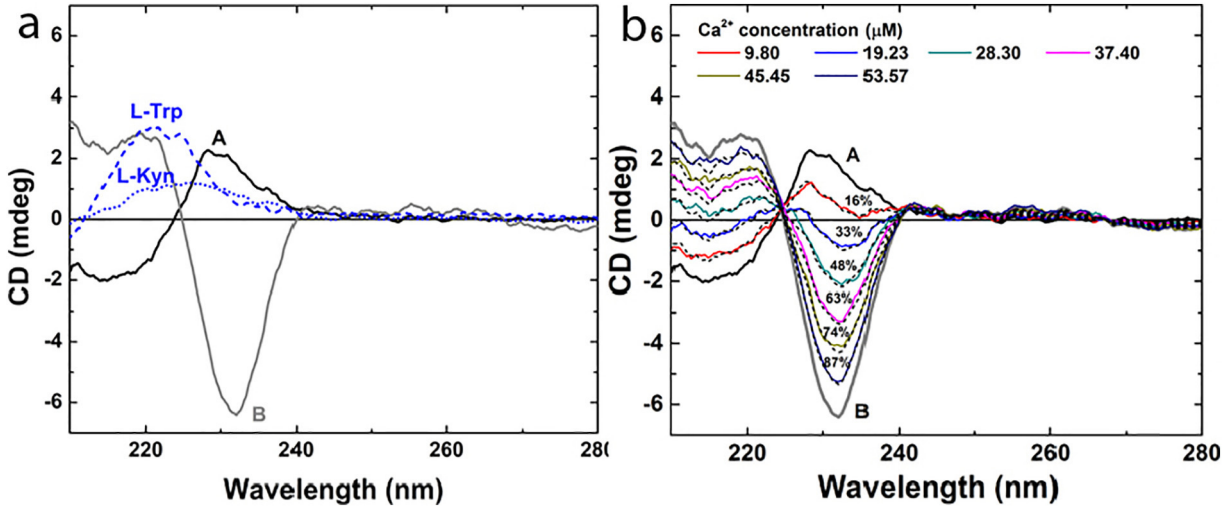

Fig. 4. CD spectra of daptomycin. (a) Spectrum A is the CD of 40 μM daptomycin with 800 μM 7:3 DOPC/DOPG in small vesicles in the absence of calcium ions. Spectrum B is the CD of 40 μM daptomycin with 800 μM 7:3 DOPC/DOPG and 97 μM $CaCl_2$. In blue color are the CD spectra of the single amino acids L-Tryptophan and L-Kynurenine in buffer solution. (b) CD spectra of 40 μM daptomycin with 800 μM 7:3 DOPC/DOPG at Ca^{2+} concentrations between 0 and 103 μM. Each spectrum can be fit by a linear combination of A and B spectra (dotted line) with the percentage of B indicated.
(Reproduced from ref. [72].)

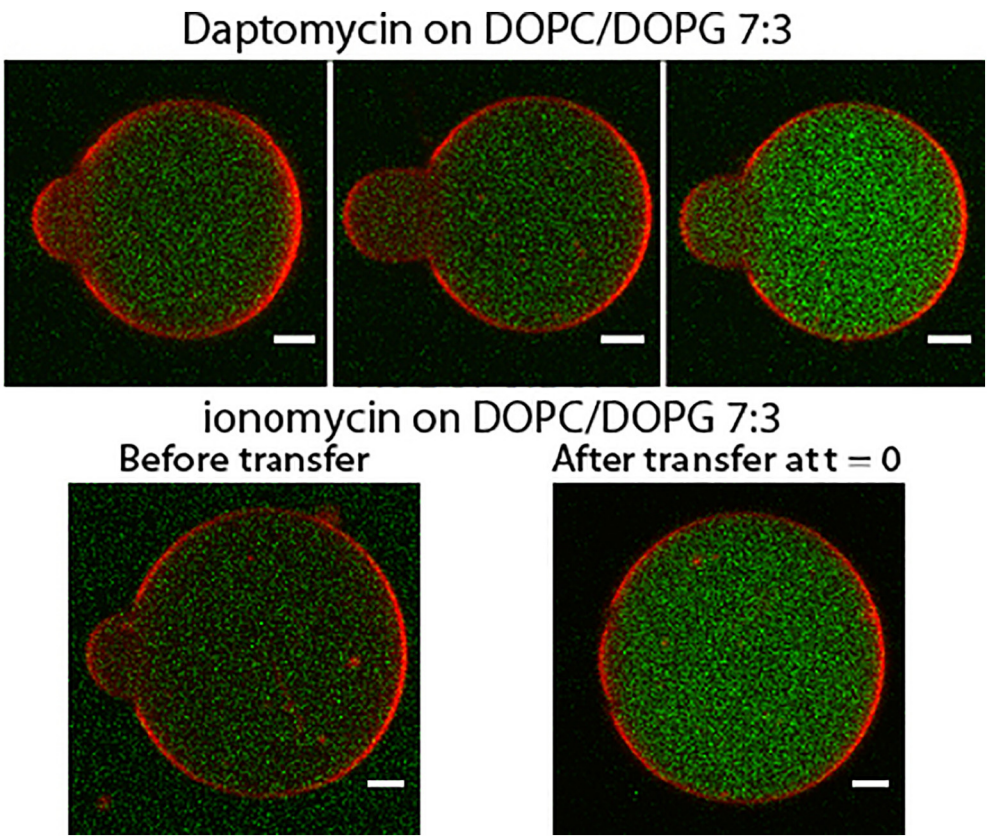

Fig. 5. Micropipette aspirated giant unilamellar vesicles (GUVs) made of 7:3 DOPC/DOPG contained calcium indicator Flou-4. (Top) The outside solution contained 1 mM Ca^{2+} and 1 μM daptomycin. (Bottom) the outside solution contained 1 mM Ca^{2+} and 0.2 μM ionomycin. Time sequence is from left to right. The induced ion leakage through the GUV membrane caused the Flou-4 fluorescence (green color), but Flou-4 did not leaked out in either case. Scale bar = 5 μm. Note that daptomycin binding first caused the GUV membrane area increase (the lengthening of the protrusion in the micropipette in the 2nd image) without ion leakage, then Ca^{2+} leaked-in in the third image. In comparison ionomycin caused Ca^{2+} leakage instantaneously.
(Reproduced from ref. [88].)

pore formation. In fact Pogliano et al. in 2012 [67], have done a much more detailed time course investigation on the effect of daptomycin to *B. subtilis*. Pogliano et al. [67] pointed out that the lysis that caused molecular leakage into *B. subtilis* was due to the activation of autolysins, whereas *S. aureus* did not exhibit lysis (i.e., no leakage to sytox green) in the presence of daptomycin. The leakage of propidium iodide into *B. subilis* shown by Seydlova et al. [91] is similar to the leakage of calcein into *E. coli* spheroplast shown in Fig. 6C above. Seydlova et al. [91] also measured ion conductance through a PG bilayer by an applied voltage at daptomycin concentrations two orders of magnitude higher than typical single-channel conductance measurement [26–28,32,92]. The ion conduction data did not show any evidence of discrete pore formation, but they interpreted the observed complicated jumps in conductance level as due to discrete pores of various sizes, disregarding previously established standard methods for single-channel ion conductance analyses [26–28,32,92].

5. Colistin (polymyxin E) and polymyxin B

Polymyxins consist of a cyclic heptapeptide with a tripeptide side chain acylated at the N terminus by a fatty acid. Among the polymixin family, i.e., polymyxin A-E, only polymyxin B and polymixin E (colistin) have been used in clinical practice. According to a 2005 review by Falagas et al. [93], colistin was initially used therapeutically in Japan and in Europe during the 1950s and in the United States in the form of colistimethate sodium in 1959. However, the intravenous formulations of colistin and polymyxin B were gradually abandoned in most parts of the world in the early 1980s because of the reported high incidence of nephrotoxicity. It is the emergence of bacteria resistant to most classes of commercially available antibiotics and the shortage of new antimicrobial agents with activity against Gram-negative microorganisms that led to the reconsideration of polymyxins as a valuable therapeutic option.

So far the understanding of their membrane-active mechanism is rather limited. One theory proposes that the electrostatic interactions between the cationic polypeptide and anionic lipopolysaccharide (LPS) molecules on the surface of the outer membrane of the Gram-negative bacteria will lead to derangement of the cell membrane, i.e., polymyxin displaces Mg^{2+} and Ca^{2+} from the phosphate groups of LPS that act as membrane stabilizers, leading to disruption of the outer membrane and to the loss of cellular contents, thus killing the bacterium. This process was thought to be independent of the entry of polymyxins into the cell [93–95]. However, a more recent study with a fluorescence labeled polymyxin on *Klebsiella pneumoniae* seemed to indicate the entry of the polypeptide into the cell [96].

The direct study of polymyxin-membrane interactions so far includes only investigations with lipid vesicles, with and without LPS. The studies did show polymyxin binding with LPS and polymyxin causing leakage of carboxyfluorescein from lipid vesicles [97]—however, the majority of these studies were performed with polymyxin concentrations 1 or 2 orders of magnitudes higher than their MIC values which are $\lesssim$1 μM. There was also an NMR study of the molecular configuration of polymyxin bound with LPS in solution [98]. We know from our experience with other AMPs that more sophisticated experiments are needed in order to deduce the membrane-active mechanism of colistin and other polymyxins.

6. Discussion

Bacterial resistance to daptomycin was noticed not long after its clinical use. The bacterial mutations that altered susceptibility to daptomycin appeared to directly affect the membrane lipid composition [64,99,100]. Both the decrease of PG synthesis or the increase of conversion of PG to lysylphosphatidylglycerol found in the resistant mutants of *Staphylococcus aureus* [101] and *Bacillus subtilis* [102] led to reduced daptomycin activity. Recently Goldner et al. [4] further demonstrated that loss-of-function mutations in PG synthase (*pgsA2*) and the loss of membrane PG were necessary and sufficient to produce high-

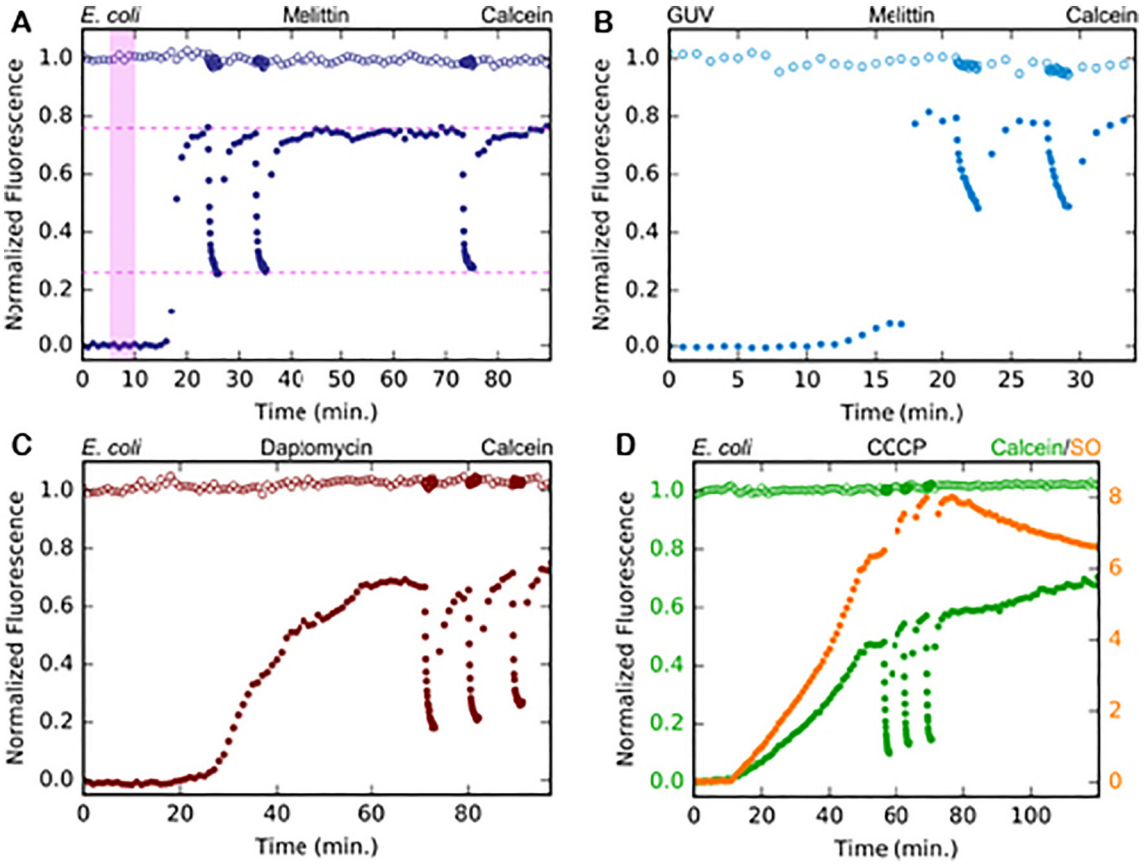

Fig. 6. (A) Shows the introduction of melittin from 5 to 10 min (pink stripe) caused calcein to abruptly leak into *E. coli* spheroplast. The pattern is closely similar to the leakage into a GUV shown in (B), consistent with pore formation by melittin. In contrast daptomycin first caused membrane depolarization by ion leakage then the spheroplast membrane gradually became leaky to calcein (C). The effect of daptomycin is closely similar to the effect of CCCP (D) which was experimented with two different dye molecules, i.e., calcein and sytox orange (SO). (Reproduced from ref. [22].) We note that Randall et al. [90] found no effect of daptomycin on *E. coli* spheroplast by susceptibility.

level resistance to daptomycin in *Corynebacterium striatum*.

We have found that daptomycin, in the presence of Ca^{2+} and PG membranes, forms a dimeric complex $Dap_2Ca_3PG_2$. Because this complex has a well defined CD spectrum and specific stoichiometric ratios [72], we have reasons to believe that the complex has a well defined structure. Resolving this structure is the key for understanding the molecular mechanism of daptomycin.

As we discussed above, daptomycin appears to function as transient ionophores in the target membrane. An ionophore (also called ion carrier) embedded in a membrane can bind ions from solution and reversibly release bound ions into the solution at the membrane boundary. It can also randomly move between two sides of the membrane. As a result, there is a time-averaged net transport of ions across the membrane by ionophores from the side of high concentration to the side of low concentration. The efficiency of an ionophore depends on its mobility. It is possible that only the small aggregates of the dimeric daptomycin complex are sufficiently mobile to function as ionophores. We have seen that daptomycin aggregation in membrane grows with time and very large aggregates eventually exit from the membrane (called the lipid extracting effect) [103]. These observations are more or less consistent with the idea that daptomycin forms transient ionophores in membranes.

Daptomycin belongs to a family of N-acylated cyclic lipopeptides that require calcium ions for antibacterial activity. The family members, varying from 10- to 13-amino-acid rings in structure, are thought to have discrete modes of action, targeting either cell wall biosynthesis or cell membrane integrity [12]. Thus resolving the membrane-active mechanism of daptomycin may open up the potential utility of a family of unique antibiotics.

Declaration of competing interest

No financial interest is involved in the publication of this article.

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