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Biochimica et Biophysica Acta 1758 (2006) 1215–1223

BIOCHIMICA ET BIOPHYSICA ACTA

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Review

Mode of action of the new antibiotic for Gram-positive pathogens daptomycin: Comparison with cationic antimicrobial peptides and lipopeptides

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Received 2 December 2005; received in revised form 1 February 2006; accepted 2 February 2006
Available online 3 March 2006

Abstract

With the steady rise in the number of antibiotic-resistant Gram-positive pathogens, it has become increasingly important to find new antibacterial agents which are highly active and have novel and diversified mechanisms of action. Two classes will be discussed here: the cationic antimicrobial peptides, which are amphiphilic in nature, targeting membranes and increasing their permeability; and lipopeptides, which consist of linear or cyclic peptides with an N-terminus that is acylated with a fatty acid side chain. One member of the cyclic lipopeptide family, the anionic molecule daptomycin, has been extensively studied and is the major focus of this review. Models will be presented on its mode of action and comparisons will be made to the known modes of action of cationic antimicrobial peptides and other lipopeptides.

Keywords: Daptomycin; Lipopeptide; Cationic peptide; Gram-positive pathogen; Mechanism of action

Contents

1. Introduction

The growing prevalence of antibiotic-resistant bacteria has increased the complexity of anti-infective therapies being administered in hospitals, nursing homes, and dialysis centres worldwide. While the phenomenon of resistance is not new, being first proposed by Sir Alexander Fleming with regards to penicillin more than 60 years ago, it has become of increasing concern as more and more antibiotics are rendered ineffective. Moreover, resistance now includes potent antibacterial agents which are used as a last resort, including methicillin and vancomycin. Approximately 30% of hospital strains of enterococci are currently vancomycin-resistant and nearly half of the infections due to *Staphylococcus aureus* are methicillin-resistant. Given this situation,

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0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.02.009

there has been an urgent need to develop new bactericidal agents which target resistant Gram-positive pathogens. A number of new antibiotics have recently been approved by the FDA or are in advanced development to try to meet this demand [1–3]. Some examples include tigecycline (sold commercially as Tygacil, by Wyeth-approved by the FDA in June 2005) which is the first in a new class of agents, the glycylcyclines, that possesses activity against key Gram-positive and Gram-negative bacterial pathogens and functions by inhibiting protein synthesis [4]. Another example includes the cyclic lipopeptide daptomycin which has been found to be an effective antimicrobial agent against methicillin-resistant *Staphylococcus aureus* (MRSA), as well as vancomycin-susceptible strains of enterococci (in vitro), *Streptococcus pyogenes*, *S. agalactiae*, and *Strephtococcus dysgalactiae* [5–10]. Finally, a number of cationic antimicrobial peptides are in various stages of development including MX-226, a derivative of bovine indolicidin that is in phase IIIb clinical trials for preventing catheter colonization and decreasing tunnel infections [11–13].

A key to future developments is to understand the mode of action of these antibacterial agents. Several excellent recent reviews describe what classes of antimicrobial agents are currently being marketed [1,14,15] and how they function [3,16–18]. Here, we will focus only on two classes: cationic antimicrobial peptides and lipopeptides.

2. Mechanism of action of cationic antimicrobial peptides

Cationic antimicrobial peptides are widespread throughout the animal and plant kingdoms and play a fundamental role in innate immune defences, both through direct antimicrobial activity and through immunomodulatory effects, in fending off a wide range of microbes—from bacteria to viruses. They are ubiquitous in nature and have remained effective defensive weapons, displaying little to no resistance effects [3,19]. In fact, only a few resistant species have been found: bacteria of the *Burkholderia*, *Morganella* or *Serratia* genuses have outer membranes that have reduced negative charge on their surface lipopolysaccharides (LPS), while under the appropriate environmental conditions, others modify their LPS through the two component regulators PhoPQ and PmrAB [19]. Other species, such as, for example, *Porphyromonas gingivalis*, secrete proteases which degrade the peptides [19]. Most of the resistance mechanisms encountered only have a moderate (2- to 4-fold) increase on the minimal inhibitory concentration (MIC) [12]. Consequently, these molecules are of great interest and are being developed as a new class of anti-infective agents.

Despite the diversity in the amino acid sequence and the structural classes (i.e., β-sheets, α-helices, loops and extended structures [12]) of antimicrobial peptides, with over 700 known to date, they all share a common three-dimensional arrangement. They fold into amphiphilic molecules, with one face being hydrophobic, while the other is charged [3]. Given this arrangement and the composition of bacterial membranes, cationic antimicrobial peptides function predominantly after directly binding to the lipid bilayer. A model which can be used to account for the initial interactions of most antimicrobial peptides with membranes has been termed the Shai–Matsuzaki–Huang model [20–24], illustrated in Fig. 1. The peptides start off being unstructured in solution (Fig. 1a). Upon interaction with the membrane, they adopt a three-dimensional structure (e.g., α-helix, β-sheet) such that the molecule becomes amphiphilic, with the positively charged side interacting directly with the lipid headgroups (Fig. 1b). The peptide then integrates into the outer half of the membrane, leading to thinning of the outer leaflet (Fig. 1c). Evidence for this thinning has recently been demonstrated using atomic force microscopy [25] and X-ray diffraction [26,27]. Following this step, channel formation can occur although this portion of the process is more controversial (Fig. 1d). A number of different models have been proposed for this, namely the barrel-stave model, the carpet model, the toroidal pore model, and the micellar aggregate channel model [28,29]. The appropriateness of each model depends on the peptide [30–37], as well as properties of the lipids (i.e., phase, elasticity, hydrophobic chain length, hydration, etc.) [38–42]. Finally, the bacterial cells are killed in one of a number of ways, examples of which

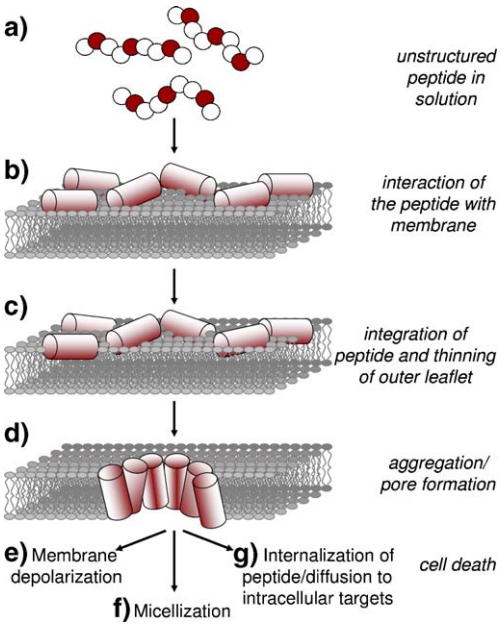

Fig. 1. Model for the mechanism of action of cationic antimicrobial peptides. The unstructured peptides (a) adopt secondary structure upon interaction with the bacterial membrane, and particularly anionic phospholipids. (b) This structure can consist of β-sheets, which are stabilized by 2–3 disulphide bonds, amphiphilic α-helices, extended molecules, β-structured loops stabilized by 1 disulphide, or mixed structures [11,19]. (c) Subsequently, the peptides are integrated into the outer leaflet straddling the membrane interface between the headgroups and the acyl chains, leading to a thinning of the bilayer. (d) This is followed by the formation of a formal or informal channel that is differently depicted as occurring via a barrel-stave mechanism, a carpet mechanism, the formation of a toroidal pore, or the formation of a micellar aggregate channel [28,29]. Finally, bacterial cells are killed by either membrane perforation (depolarization), the translocation of the peptide across the membrane leading to the cationic peptides attacking intracellular targets, or membrane disintegration (micellization), and can involve a mixed multi-hit mechanism with several of these events occurring with similar efficiency.

include: (i) membrane depolarization [43]; (ii) damage of intracellular processes such as macromolecular synthesis [44], degradation of cell walls [45], or modification of the lipid composition in the membrane bilayer [22]; or (iii) in extreme cases, formation of micelles, leading to cell leakage [21].

Despite the relatively high peptide concentrations needed for activity (usually μM) [19], the unique mechanism of action of cationic peptides makes them interesting starting points for development of new antimicrobial agents. Since the mode of action of these peptides relies on their interaction with the membrane bilayer, via charge–charge and hydrophobic interactions, resistance is curtailed because it would be too costly or require too many mutational events for a microbe to change the composition or organization of its lipids in order to weaken these interactions. In addition, the large variability in peptide sequence found for these peptides ensures that there is no unique recognition site for protease cleavage. Another important factor in curbing resistance is the existence of secondary targets, i.e., although cationic peptides preferentially bind to specific targets, they can also interact with other targets in the bacterial cell and affect processes such as cell-wall synthesis or degradation, cell division, or macromolecular synthesis [12]. Finally, it should be noted that multicellular organisms often attack bacteria by using more than one cationic peptide, thereby minimizing the chances of antibiotic resistance.

3. Mechanism of action of lipopeptides

Lipopeptides consist of a linear or cyclic peptide sequence, with either net positive or negative charge, to which a fatty acid moiety is covalently attached to its N-terminus. They are a class of antibiotics which are highly active against multi-resistant bacteria. Some lipopeptides also display anti-fungal activity [46–49]. Recently, a series of synthetic lipopeptides derived from non-membrane active peptides conjugated to palmitic acid [48] were synthesized and characterized in order to try to understand which features are key to lipopeptide antibacterial activity [49]. In addition, a number of lipopeptides consisting of cationic amphiphilic peptides with an acetylated N-terminus (C8–C18 fatty acid chain length) have been characterized [50–55]. Of these, members of the polymyxin family have been studied extensively [29,56–59] and will not be further considered here.

In the anionic lipopeptide class, the first naturally occurring member to be discovered was amphomycin over fifty years ago [60]. Additional members of this class of compounds include crystallomycin [61,62], aspartocin [63–66], glumamycin [67–69], laspartomycin [70], tsushimycin [71,72], and, by far, the most studied, daptomycin [73–77]. Because of their unique composition, they function in a manner which is atypical for most antibacterial agents. In particular, they neither inhibit cell wall synthesis by interacting with ribosomal subunits nor do they inhibit protein synthesis [1]. Rather, they are believed to target and bind to the bacterial membrane directly [55], and cause rapid depolarization of the antibacterial membrane potential [48,78]—in a manner somewhat reminiscent of the cationic peptides, described above. Since this mechanism is distinct from those of other antibiotics currently on the market, only a few rare cases of resistance to anionic lipopeptides (in this case, daptomycin) have been reported to date [79,80]. In fact, resistance is generally rare against all lipopeptides, including echinocandins [81]. Moreover, since the distribution of minimal inhibitory concentrations (MICs) is unimodal, a well-defined resistance mechanism has been proposed to be unlikely [82]. In addition, the lack of use of these antibiotics in animal husbandry has afforded fewer opportunities for resistance development in notoriously intractable species like the enterococci, in contrast to the tetracyclines, macrolides, glycopeptides and quinolones, which have been used extensively [3]. Finally, most anionic lipopeptides possess Asp–Gly segments in their amino acid sequence, making them prone to chemical reaction and degradation under physiological conditions [83]. This also serves to prevent the development of resistance as the antibiotics can be readily eliminated from the environment.

Though much remains to be understood in the mechanism of action of all lipopeptides, a few key properties have emerged. For one, a number of lipopeptides tend to oligomerize. Evidence for this has been demonstrated in the crystal structure of tsushimycin [84], which is found to form dimers, as well as micelles constituted of 30–40 antibiotic molecules in a Ca^{2+}-containing solution [85]. Moreover, the line-broadening of the NMR signals in ^{1}H spectra of daptomycin in the presence of 1 molar equivalent of Ca^{2+} suggests that this lipopeptide can form aggregates [86–88]. Avrahami and Shai [48] found that all of the lipopeptides they investigated, designated as PA-K_4X_7W, where X=Gly, Ala, Val, or Leu and PA refers to the palmitic acid tail, were capable of aggregation, though the oligomerization state was not determined exactly. Interestingly though, those peptides that were already significantly hydrophobic (X=Val) without the presence of the palmitoyl moiety, or highly hydrophobic once conjugated to palmitic acid (X=Leu), displayed no bactericidal activity against either Gram-positive (*S. aureus*, *B. subtilis*) or Gram-negative (*P. aeruginosa*, *A. baumannii*, *E. coli*) bacteria. The authors therefore suggested that although formation of micelles is important, the oligomers should be capable of dissociating in order to ultimately interact with the bacterial membranes.

The second important property of lipopeptides is their ability to interact with membranes via their lipid tails. This applies to all peptides investigated to date, regardless of the net charge of the peptide moiety. For cationic peptides, which given their amphiphilic nature can interact quite strongly with the negatively charged bacterial membrane, the addition of a lipid tail of appropriate length (typically C10–C12) generally tends to increase the bactericidal activity. This can be due to either an increase in the affinity of the lipid tail for the hydrocarbon chains or as a result of the stronger interaction of the cationic peptide with the lipid headgroups. It has been shown that if the lipopeptide consists of only one hydrocarbon tail, the binding of the lipopeptide to the membrane is only slightly increased [54]. For example, for MSI-843,

Thennarasu et al. [55] showed that the insertion of the N-terminal octyl group and aliphatic side chains into a POPC bilayer only contributes less than a third of the binding enthalpy. When peptides are acetylated with two hydrocarbons chains, then the interaction is sufficiently strong to anchor the peptide firmly in the membrane [54]. On the other hand, the addition of a fatty acid modification to a cationic peptide has been shown to promote the formation of α-helical secondary structure [51,53]. For MSI-843, for instance, the remaining two thirds of the binding enthalpy arises from α-helix formation [55]. Since the formation of secondary structure is essential for interaction of these amphiphilic molecules with the membrane, lipidation of cationic peptides contributes to increased activity by driving the cationic peptide moiety into the correct conformation. For lipopeptides with net negative charge, the situation is quite different. In this case, divalent cations (e.g., Ca^{2+}) are needed to lock the lipopeptide into an amphiphilic conformation. Indeed, both daptomycin [86] and tsushimycin [84], whose three-dimensional structures have been solved by NMR and X-ray crystallography respectively, have been found to display a predominantly hydrophilic side on one face of the structure and a hydrophobic side on the other. In both cases, calcium may also play a role in strengthening the interaction between the peptide and the membrane bilayer (see below).

As mentioned previously, the exact mechanism of action of this family of peptides and, in particular, of anionic lipopeptides remains to be determined. It has been suggested that tsushimycin, for example, could function by either [85]: (a) inhibiting phospho-N-acetylmuramoyl-pentapeptide transferase (MraY), required in the biosynthesis of the bacterial cell wall; (b) binding with lipoteichoic acids, which are on the bacterial cell surface; or (c) dissipating the membrane potential, i.e., depolarization. Most of these proposals are based on the crystal structure [84]. For example, the distance between the Ca^{2+} ions in the tsushimycin dimer is ca. 9.2 Å, a distance similar to the length of the repeating phosphate groups in lipoteichoic acids. Similar mechanisms have been proposed for the mode of action of daptomycin: (1) inhibition of lipoteichoic acid biosynthesis in the presence of calcium ions [89], but this has been disputed [78]; or (2) binding of calcium, followed by a change in conformation [86], allowing the peptide to insert deeper into the membrane bilayer. This in turn leads to membrane depolarization and may result in cell death [78,86,90]. To try to understand more about how anionic lipopeptides function, we will now focus on a discussion of daptomycin and examine structural and functional data for this peptide and suggest more detailed models on its mode of action.

4. Daptomycin

A large number of papers and reviews have been written on daptomycin (for recent work see [3,14,15,78,91,92]). The general consensus in the literature at present is that daptomycin displays rapid bactericidal activity by binding to the cytoplasmic membrane in a calcium-dependent manner (Fig. 2a–c) and oligomerizing in the membrane (Fig. 2d), leading to an efflux of potassium from the bacterial cell (Fig. 2e). This in turn leads to cell death, as this loss of potassium leads to dysfunction of macromolecular synthesis [78]. This mechanism is different from the one proposed for β-lactams, as it does not depend on cell lysis [78]. Recent data on the interaction of daptomycin with divalent cations and model membranes obtained in our laboratories or through collaboration leads us to propose a revised mechanism.

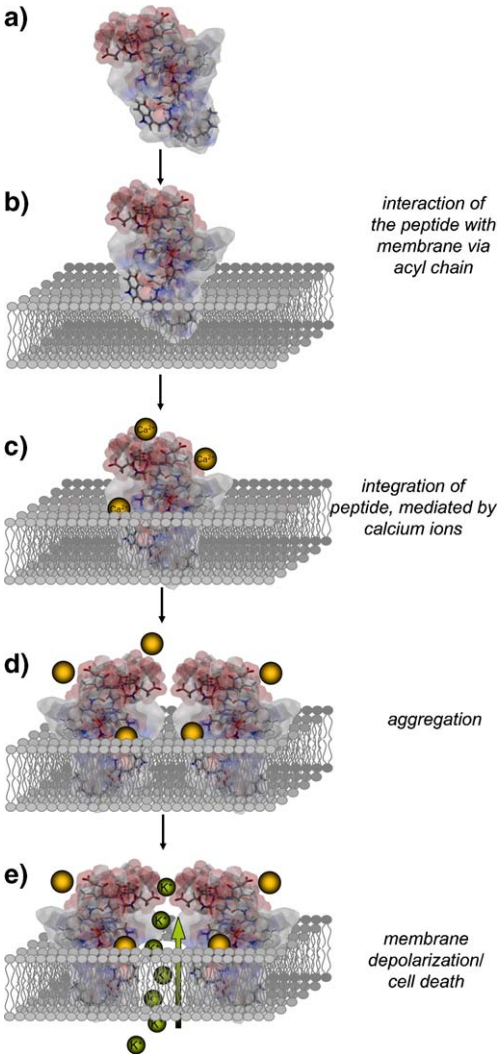

Fig. 2. Previous mechanism of action of daptomycin, as reported in the recent literature (see text): (a, b) Daptomycin interacts with the bacterial membrane via its lipid tail and inserts partly. (c) In the presence of Ca^{2+} which bridges between the anionic daptomycin and the anionic headgroups of bacterial outer leaflet lipids, the lipopeptide inserts more deeply into the membrane and (d) forms aggregates. Finally, in panel e, cell death occurs via membrane perforation (assessed as depolarization) or some other membrane-associated event.

4.1. Interaction with divalent cations

Daptomycin absolutely requires calcium for activity [8,93,94]. Recently, Jung et al. [86] showed that calcium (as Ca^{2+} ions) is needed to trigger two structural transitions in

daptomycin. In a first step, calcium binds to daptomycin in solution, resulting in a more tightly defined family of structures. In fact, if one clusters structures of the apo-form of daptomycin (Protein Databank entry: 1T5M.pdb) which have an overall distance between them of 1.5 Å or less [95], one finds 9 different families of structures. If one uses the same clustering algorithm for the calcium-bound structures (1T5N.pdb), only 7 families of structures are found. This is consistent with the suggestion that Ca^{2+} is needed to lock daptomycin into an active conformation. Indeed, the need for ions to lock a flexible peptide into a functional conformation is not uncommon and has also been observed for tsushimycin [84,85]. In addition, Ca^{2+} is needed to render daptomycin more amphiphilic, suggesting that Ca^{2+} promotes insertion of daptomycin into membrane bilayers, in accordance with what has previously been proposed (Fig. 2c). The second structural transition observed in daptomycin [86] requires the presence of both Ca^{2+} and lipids with negatively charged headgroups (e.g., phosphatidyl glycerol). Although the exact nature of this change remains to be characterized structurally, the effect of daptomycin on 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-rac-1-glycerol (POPC/POPG) (1:1) lipid bilayers in the presence of Ca^{2+} is to perturb the membrane further and induce leakage.

Structural investigations of daptomycin by NMR [86–88] have all shown that significant line-broadening occurs when Ca^{2+} is added in a 1:1 Ca^{2+}/daptomycin molar ratio, suggesting that addition of Ca^{2+} leads to oligomerization. This has recently been confirmed using equilibrium sedimentation experiments, where upon addition of one molar equivalent of Ca^{2+}, an aggregate consisting of 14–16 daptomycin molecules was found. Samples were prepared using a 2.5 mM daptomycin solution (with KCl, pH 7.0) to which 0.25, 0.5, 0.75, 1, and 2.5 equivalents of $CaCl_2$ was added. The experiments were performed using a Beckman XLI analytical ultracentrifuge, at 40, 45, and 50 KRPM. The data were analyzed by curve-fitting to an equation describing the sedimentation equilibrium for a monomer (Ho, S.W., Okon, M., Calhoun, J.R., Lear, J.D., Jung, D., Hancock, R.E.W., Scott, W.R.P. and Straus, S.K., unpublished results). This evidence, as well as ^{13}C NMR data, indicates that the addition of Ca^{2+} induces the formation of micelle-like structures. The daptomycin molecules would be arranged such that the lipid tails point inwards, similarly to observations for tsushimycin in the crystal [84]. The calcium ions would help hold the charged sidechains of daptomycin from different monomers together. Whether this interaction always occurs preferentially between specific residues or rather between different residues is currently being investigated. Presumably, as with the synthetic lipopeptides studied by Avrahami and Shai [48,49], these micelles should be able to dissociate when they come into close contact with the bacterial membrane, so that daptomycin can insert and perturb the membrane. The need for large concentrations of Ca^{2+} (ca. 1000-fold more than the concentration of daptomycin) for activity supports the hypothesis that Ca^{2+} binds weakly to daptomycin such that the micelle formed can readily dissociate.

Studies with other divalent cations such as Mn^{2+}, Mg^{2+}, Cu^{2+} and Ni^{2+} (Jung, D. and Hancock, R.E.W., unpublished results) have shown that replacing Ca^{2+} with Mn^{2+} results in a 32-fold increase in the MIC (in μg/ml), whereas using either Mg^{2+}, Cu^{2+} or Ni^{2+} increases the MIC by greater than 64-fold. Furthermore, it was found that the addition of Mn^{2+} or Mg^{2+} to daptomycin in the presence of POPC/POPG (1:1) liposomes does not lead to calcein leakage, but does result in some lipid flip-flop. In fact, for both Mn^{2+} or Mg^{2+}, the flip-flop measured was approximately half of what was observed when Ca^{2+} was added and absolutely required the presence of lipids with negatively charged headgroups. Finally, addition of a 1:1 equivalent of Mg^{2+} to daptomycin did not change the structure of this antibiotic, relative to the apo-form. Equilibrium sedimentation results showed that at a 1:1 molar ratio of Mg^{2+} to daptomycin, only a very small fraction of daptomycin oligomerized. Instead, a 2.5:1 Mg^{2+} to daptomycin ratio was needed to achieve the same degree of aggregation as was obtained for a 1:1 ratio of Ca^{2+} to daptomycin. Taken together, these data indicate the following: (1) that Ca^{2+} can bind better to daptomycin compared to any of the other cations; (2) that the change in conformation observed in going from the apo-structure of daptomycin to the Ca^{2+}-bound form [86] is associated with micelle formation; and (3) that the ability of daptomycin to induce leakage plays a more important role in accounting for its antibacterial activity than its ability to promote lipid flip-flop. This in turn may imply that the formation of micelles is important to deliver daptomycin to the bacterial membrane in the "correct" conformation. It is also consistent with the suggestion that daptomycin is not transported across the bacterial membrane via a flip-flop mechanism but acts by perturbing the membrane on the surface. It should be noted that recent work has shown that the interaction of calcium ions with negatively charged lipid headgroups is stronger than those of magnesium ions [96], implying that Ca^{2+} may also be more effective in acting as a bridge between daptomycin and lipid headgroups than magnesium ions. Again the need for high concentrations of calcium for activity supports the hypothesis that Ca^{2+} is important for daptomycin binding to bacterial membranes.

4.2. Interaction with bacterial membranes

In addition to calcium, daptomycin interactions with lipid membranes depend substantially on the presence of lipids with negatively charged headgroups. Although lipid flip-flop occurs in both neutral membranes consisting of POPC alone and charged membranes of POPC/POPG (1:1) membrane leakage only occurs for POPC/POPG (1:1) bilayers (in the presence of Ca^{2+}). Moreover, liposome fusion can only be induced by daptomycin when POPG is present in the vesicle.

Our recent unpublished studies using differential scanning calorimetry in model membranes of DiPoPE have shown that daptomycin can induce positive curvature strain. Adding daptomycin alone to membranes consisting of DiPoPE shifted the transition temperature from 43.4 °C (for pure lipid) to

45 °C. Addition of 5 mM Ca^{2+} in the presence of daptomycin, which on its own favors hexagonal lipid phase formation, resulted in a main transition peak at 42 °C. These results are analogous to what was recently reported for MSI-843 [55] and a number of other lipopeptides [54] and indicate that daptomycin perturbs the membrane strongly. Recently, the cationic antimicrobial peptide magainin 2 was shown to promote curvature strain and result in detergent-like properties [97]. This may be relevant for daptomycin's mechanism of action as well.

4.3. Mode of action of daptomycin: revised model

Taken together, the recent findings presented above lead us to propose a new model for the mode of action of daptomycin, as illustrated in Fig. 3. In a first step, daptomycin aggregates in solution in the presence of a minimum of 1:1 calcium to daptomycin molar ratio. The presence of Ca^{2+} results in a change in the conformation of daptomycin to one which is better defined than in the apo-form (Fig. 3a). Support for this calcium-induced change in conformation comes from NMR and CD data [86]. It should be noted that studies [88] performed under different sample conditions to those used by Jung et al. [86] have shown that no major structural changes occur in the presence of calcium. This may be due to a dependence of the peptide structure on "solvent", a fact which is not uncommon for such molecules. Indeed, the apo-structure of daptomycin reported by Ball et al. [88] is different to that reported by Rotondi and Gierasch [87], where the sample contained 10 mM sodium phosphate buffer in addition to the 10% D_2O/90% H_2O, pH 5 used by Ball et al.. Despite these discrepancies, all of the studies mentioned agree that daptomycin readily forms aggregates, particularly in the presence of calcium, where it forms micelles (see above). In order for daptomycin to interact with the bacterial membrane, this micellar structure may need to dissociate (Fig. 3b). Daptomycin then inserts into the membrane, a process facilitated by calcium, which binds strongly to phosphatidylglycerol headgroups (Fig. 3c). This insertion may be accompanied by a second conformational transition, as described by Jung et al. [86]. At this point, daptomycin induces positive curvature strain on the lipids (Fig. 3d). It may also oligomerize in the membrane, a fact which is as of yet undetermined. As a final step, leakage occurs, leading to cell death (Fig. 3e). It is also possible that daptomycin aggregation in the membrane would interfere with membrane-associated processes including synthesis of cell wall components, energetics, cell division, etc.

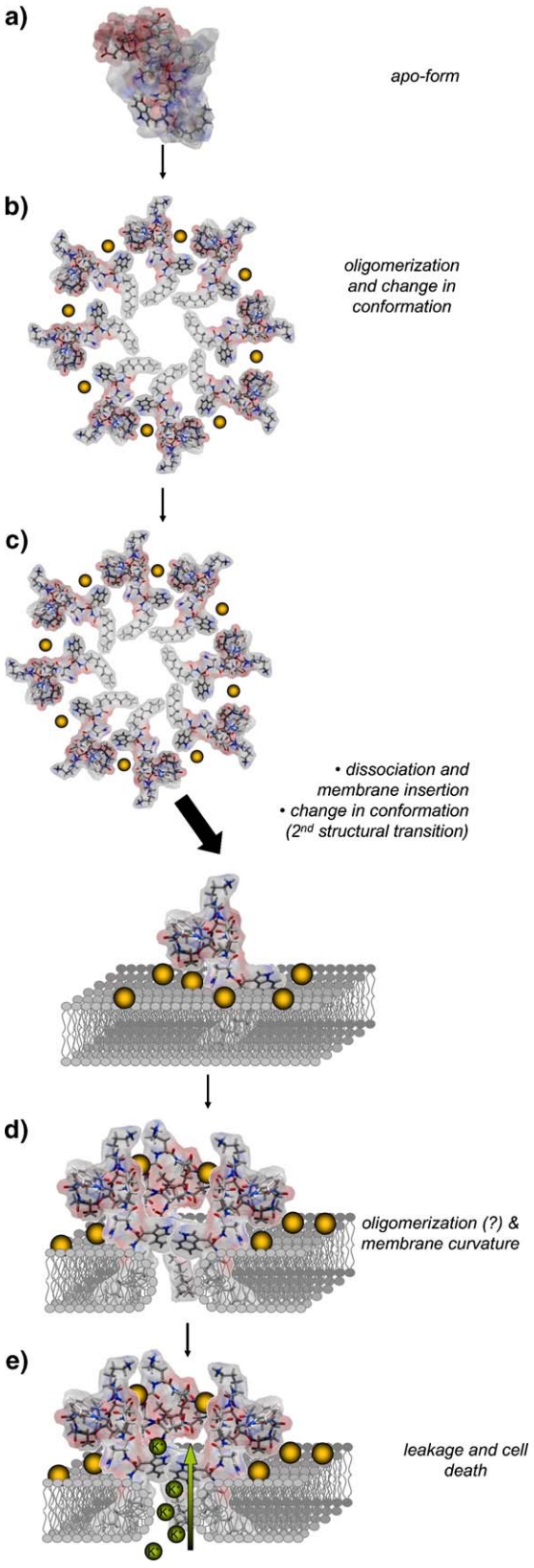

Fig. 3. Revised mechanism of action of daptomycin: (a) without calcium, daptomycin adopts a structure which is reasonably well defined but not highly amphiphilic. Once a 1:1 calcium to daptomycin molar ratio is reached, the lipopeptide oligomerizes (b) to form a 14–16 mer and most likely arranges itself into a micelle. This process is accompanied by a change in conformation. (c) Once daptomycin comes into close proximity with the bacterial membrane, the multimer dissociates, and daptomycin inserts into the bilayer. This is accompanied by a second structural transition [86], the exact nature of which remains to be determined. (d) Insertion of daptomycin into the membrane is accompanied by the induction of positive membrane curvature. Oligomerization in the membrane may occur. Finally, (e) bacterial cells are killed by membrane perforation (assessed as depolarization) or some other membrane-associated event.

5. Conclusions and perspective

One should note that many of the findings for daptomycin (peptide aggregation, association with membranes leading to a conformational change in the peptide and alteration of lipid phase transitions, lipid flip-flop at lower concentrations, induction of membrane leakiness at high concentrations, induction of curvature strain) and many of the features of this mechanism (Fig. 3) are analogous to what has been observed for cationic peptides. Thus, it seems likely that Nature has found two chemical solutions to bring about analogous mechanisms of action, namely cationic peptides with lipophilic domains (either lipid tails or hydrophobic amino acid patches) or anionic lipopeptides that are absolutely dependent on the presence of Ca^{2+} for their action. It is proposed that the divalent Ca^{2+} ions serve to "convert" the negatively charged residues on the anionic lipopeptides into pseudo positive charges thus promoting interaction with negatively charged lipids (which are also required for the action of most cationic antimicrobial peptides). Having gained some insight into Nature's design plan, we propose that antimicrobial designers will be well equipped to initiate studies to rationally design even more potent lipopeptides.

Acknowledgements

The authors would like to thank the many co-workers who were involved in different aspects of the work on daptomycin: David Jung, Jon-Paul Powers, Mark Okon, Steven W. Ho, Walter R.P. Scott, Annett Rozek, Jennifer Calhoun, James D. Lear, and Jared Silverman. SKS gratefully acknowledges the support of NSERC through a Discovery Grant and a University Faculty Award and UBC. REWH was funded by the Applied Food and Materials Network for this research and by a contract from Cubist Pharmaceuticals, and is the recipient of a Canada Research Chair.

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