Biochimica et Biophysica Acta 1788 (2009) 289–294

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Lipid domains in bacterial membranes and the action of antimicrobial agents

Richard M. Epand *, Raquel F. Epand

Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5

ARTICLE INFO

Article history:
Received 17 July 2008
Received in revised form 27 August 2008
Accepted 31 August 2008
Available online 10 September 2008

Keywords:
Bacterial outer membrane
Cell wall
Antimicrobial agent
Bacterial membrane
Cationic sterol derivative
Gram negative bacteria
Phosphatidylethanolamine
Phase separation
Phase boundary defect

ABSTRACT

There has been increasing interest in recent years in describing the lateral organization of membranes and the formation of membrane domains. Much of the focus in this area has been on the formation of cholesterol-rich domains in mammalian membranes. However, it is likely that there are domains in all biological membranes. One of the challenges has been to define the chemical composition, lifetime and size of these domains. There is evidence that bacteria have domains that are enriched in cardiolipin. In addition, the formation of lipid domains can be induced in bacteria by clustering negatively charged lipids with polycationic substances. Many antimicrobial compounds have multiple positive charges. Such polycationic compounds can sequester anionic lipids to induce lipid phase separation. The molecular interactions among lipids and their lateral packing density will be different in a domain from its environment. This will lead to phase boundary defects that will lower the permeability barrier between the cell and its surroundings. The formation of these clusters of anionic lipids may also alter the stability or composition of existing membrane domains that may affect bacterial function. Interestingly many antimicrobial agents are polycationic and therefore likely have some effect in promoting lipid phase segregation between anionic and zwitterionic lipids. However, this mechanism is expected to be most important for substances with sequential positive charges contained within a flexible molecule that can adapt to the arrangement of charged groups on the surface of the bacterial cell. When this mechanism is dominant it can allow the prediction of the bacterial species that will be most affected by the agent as a consequence of the nature of the lipid composition of the bacterial membrane.

Contents

1. Composition of bacterial membranes

There are two general motifs for the organization of bacterial membranes. These correspond to the membranes of Gram positive and of Gram negative bacteria. A major difference between these two classes of bacteria is that Gram positive bacteria have only one membrane, the cytoplasmic membrane that surrounds the cell, while Gram negative bacteria have two membranes: the cytoplasmic membrane and in addition an outer membrane. There are also other differences. While both Gram positive and Gram negative bacteria have a peptidoglycan layer on the outer side of the cytoplasmic membrane, the peptidoglycan layer is much thicker for Gram positive bacteria and helps to maintain the shape of these bacteria. Another difference is that both kinds of bacteria contain different lipopolysaccharides in their membranes, although both types have in common that they possess phosphate groups and are negatively charged. In the case of Gram positive bacteria these lipopolysaccharides are lipoteichoic acids (LTA) that are imbedded in the cytoplasmic membrane,

Abbreviations: LTA, lipoteichoic acid; LPS, lipopolysaccharide; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; DMDG, dimannosyldiacylglycerol; DSC, differential scanning calorimetry; MAS/NMR, magic angle spinning/nuclear magnetic resonance; MIC, minimal inhibitory concentration

* Corresponding author. Tel.: +1 905 525 9140x22073; fax: +1 905 521 1397.
E-mail address: epand@mcmaster.ca (R.M. Epand).

0005-2736/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2008.08.023

while in Gram negative bacteria the lipopolysaccharide (LPS) forms the major lipid component of the outer leaflet of the outer membrane. The outer membrane of Gram negative bacteria is permeable to hydrophilic molecules smaller than ~600 Da because of the presence of β-barrel proteins termed porins.

There is a large difference in the lipid composition of bacterial cytoplasmic membranes. For most bacteria the predominant zwitterionic phospholipid is phosphatidylethanolamine (PE). In general Gram negative bacteria have a higher content of PE than Gram positive bacteria. Some Gram positive bacteria have a very low content of zwitterionic phospholipids. The predominant anionic lipids in bacterial membranes are phosphatidylglycerol (PG) and cardiolipin (CL). All bacteria have at least 15% anionic lipid, but this can be either PG or CL or both and it is not dependent on whether it is a Gram negative or Gram positive organism. It is the exposure of these anionic lipids, along with LPS or LTA or peptidoglycan that provide the selectivity of cationic antimicrobial agents for toxicity against bacteria but not against mammalian cells.

2. Evidence for domains in bacterial membranes

There is evidence from chemical crosslinking of lipids that the lateral distribution of lipids is not uniform in bacterial membranes [1]. In the case of *Micrococcus luteus*, the amount of homodimers of PG and of dimannosyldiacylglycerol (DMDG), formed in intact bacteria by photoactivation of a probe, was higher when compared with PG-DMDG heterodimers [2]. It was shown that this was not a consequence of a highly asymmetric transverse distribution of these lipids, but rather a consequence of the presence of lateral domains. The distribution of lipids is altered during the cell cycle [3]. In addition to lipid miscibility, specific lipids are also involved in the formation of protein-enriched domains in membranes. An example is the formation and constriction of FtsZ rings during cell division of *Escherichia coli* that is dependent on the presence of PE [4], indicating that PE is enriched at the septum of a dividing cell.

The presence of domains in *E. coli* membranes was also shown by the existence of regions in the membrane with different order and polarity as measured using the fluorescent probes Laurdan and 1,3-diphenyl-1,3,5-hexatriene [5]. The existence of domains enriched in either PE or PG was demonstrated in both *E. coli* and *Bacillus subtilis* using lipids labeled in the *sn*-2 position with 1-pyrene decanoic acid [6]. This work showed that PE and PG sequestered into different pre-existing domains in the bacterial membrane.

There have also been studies showing the presence of membrane domains in bacteria using fluorescence microscopy imaging methods. The selective staining of septal regions has been observed in mycobacteria [7] and the uneven distribution of a lipophilic dye in *E. coli* has been used to demonstrate membrane domain formation [8]. It has been suggested that lipid domains may be important for certain regulatory functions of the cell [1,9,10]. In particular, domains of acidic lipids may be important for the initiation of chromosome replication in *E. coli* [11,12].

Formation of domains enriched in CL has attracted particular attention, in part because there is evidence that 10-nonyl acridine orange (NAO) is a cardiolipin-specific dye [13] that can be used for imaging. It has been shown that CL localizes at the polar and septal regions of the cytoplasmic membrane of *E. coli* [14,15] as well as in *B. subtilis* [16]. In addition, elevated levels of CL were found in the membrane of *E. coli* minicells that are derived from the cell poles [17]. The polar and septal targeting of CL has also been suggested to have a direct role in the targeting of an osmosensory transporter, ProP [18,19]. A recent paper using continuum mean-field analysis of membrane energetics suggests that there is a critical concentration of CL required for microphase separation [20]. This study also suggests that there is a relationship between CL domain formation and membrane curvature. Mukhopadhyay et al. [20] predict that spherical bacteria will not exhibit large-scale CL localization without variations in cell wall curvature. Curvature also explains why CL localizes to the higher curvature division sites in *E. coli* [14,15] and *B. subtilis* [16]. Another membrane lipid with high negative curvature tendency, PE, is also found in these high curvature regions at the poles of *E. coli* and *B. subtilis* [4,21].

3. Promotion of domain formation by antimicrobial compounds

Antimicrobial agents have a wide variety of chemical structures [22]. Several mechanisms have been proposed to account for their protection against infection including effects on the innate immune system [23], damage to the cytoplasmic membrane of bacteria [24], binding to DNA [25] or inhibition of specific bacterial metabolic processes [26]. These agents do not have a high specificity. Many are selectively toxic to bacteria because these organisms, unlike mammalian cells, contain exposed anionic groups. Even nisin, an antibiotic with a high degree of specificity of binding to lipid II, has recently been shown to have several modes of action [27]. Hence in many cases there is likely more than one factor contributing to the toxicity of antimicrobial agents. The membrane plays an important role in many of the mechanisms of toxicity of these agents either as a target of the antimicrobial compound, resulting in damage to the membrane or as a barrier that must be traversed by the toxic agent to gain access to an intracellular target. Mechanisms of membrane damage have generally focused on how an antimicrobial cationic agent can disrupt membrane bilayer organization by forming pores lined by both lipids and peptides [28] or by a more general "carpet mechanism" [24].

Recently, a more specific mechanism has been suggested for breaching the permeability barrier of membranes that involves the antimicrobial cationic agent inducing separation of lipid components, resulting in the clustering of anionic lipids (see Fig. 1) and possibly the formation of phase boundary defects between lipid domains [29–31]. Phase boundary defects have been suggested to be responsible for the increased leakage of liposomes at the phase transition temperature where gel and liquid crystalline domains coexist [32]. However, segregated phases or domains in membranes do not by themselves lead to toxicity, since it is believed that domains are commonly present in biological membranes. We suggest that because of the large molecular heterogeneity in biological membranes domain interfaces are stabilized by concentrating other molecules at the domain interface to lower the membrane tension. In contrast, when domains are acutely formed as a consequence of the addition of an antimicrobial agent, there is insufficient time for the membrane to rearrange to accommodate this change in organization. Such a

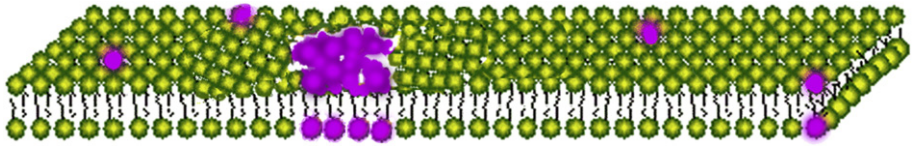

Fig. 1. Cartoon illustrating the type of lipid rearrangement that can occur on binding certain cationic antimicrobial agents. Clustering of anionic lipids (red headgroups) into a separate domain occurs as a consequence of binding to the antimicrobial agent. This causes a rearrangement of lipids around the domain, leaving defects in the membrane and recruiting anionic lipids from other locations in the membrane where they may be required for function.

mechanism may be particularly important for antimicrobial compounds that have a sequence rich in cationic residues and have conformational flexibility that can adapt to the distance between charges on the membrane surface. In addition to the increased permeability caused by the formation of phase boundary defects, the membrane lipid rearrangements caused by the clustering of anionic lipids are also likely to perturb existing domains in the membrane, adversely affecting the bacteria. The importance of induced lateral phase separation has been specifically proposed as a mechanism contributing to the antimicrobial activity of a designed alpha/beta-peptide [29], for a flexible sequence-random polymer [30], for cateslytin [31] and for an oligo-acyl-lysine (OAK) [33].

This group of four antimicrobial agents represents diverse chemical structures and conformational motifs. They are not likely to be the only compounds for which lipid segregation plays a role in the mechanism of antimicrobial action. Furthermore, clustering of anionic lipids is not likely to be the sole mechanism by which these agents act to kill bacteria, but rather it is an additional contributory factor, more important for some antimicrobial agents than for others. For example, we have also shown that the flexible sequence-random polymer can kill bacteria by encapsulating them, independent of any damage to the cytoplasmic membrane [30]. Factors that would facilitate preferential interaction with anionic lipids and the promotion of phase segregation are:

1. The presence of multiple cationic groups allowing one molecule of the antimicrobial agent to interact with several anionic lipid molecules.
2. A conformational flexibility to facilitate the adoption of a conformation in which the distance between positive charges matched the distance between anionic lipids in the bacterial membrane after clustering.
3. Being sufficiently hydrophobic to spontaneously partition to a membrane.

Two alpha/beta peptides have been synthesized having identical chemical composition but differing in the distribution of cationic groups [34]. Both of these peptides are believed to form a "14/15-helical conformation" [34]. One of these peptides, referred to as α/β-peptide I, has the cationic groups regularly distributed on the sequence so that it folds into a structure in which these groups are clustered along the length of one face of the 14/15-helix (Fig. 2). The other analog, α/β-peptide II, has the cationic residues more clustered in the primary structure so that the charges are distributed around the long axis of the helix. It is found by studying fluorescence resonance energy transfer between labeled lipids that α/β-peptide II is more effective in segregating anionic and cationic lipids than is α/β-peptide I [29]. This is in accord with the finding that α/β-peptide II is more toxic to *E. coli* [34]. Because α/β-peptide II has a less rigid structure when bound to membranes its cationic groups can adapt to the distance between negative charges on membrane lipids. The amphipathic helical α/β-peptide I would have more fixed distance between charges in the helical structure and therefore less adaptable to the membrane. Nevertheless, α/β-peptide I is equally potent to α/β-peptide II against *B. subtilis*. Presumably this toxicity of α/β-peptide I against *B. subtilis* is by a mechanism different from phase separation. Like for other cationic amphipathic α-helical antimicrobial peptides, such as magainin, the mechanism may be pore formation induced by the peptide.

The sequence-random polymers containing cationic and lipophilic subunits (Fig. 3) also possess properties that would allow them to sequester anionic lipid. They were designed and shown to be random structures in solution, but are suggested to cluster positive charges at an anionic membrane interface (Fig. 4) [35]. It was shown by differential scanning calorimetry (DSC) that one of these sequence-random cationic copolymers can sequester anionic lipids [30].

Cateslytin is a 15 amino acid peptide with 5 cationic residues in the form of Arg. The sequence of cateslytin is RSMRLSFRARGYGFR. The positive charges are not placed at regular intervals along the sequence, suggesting that the peptide will not form amphipathic structures,

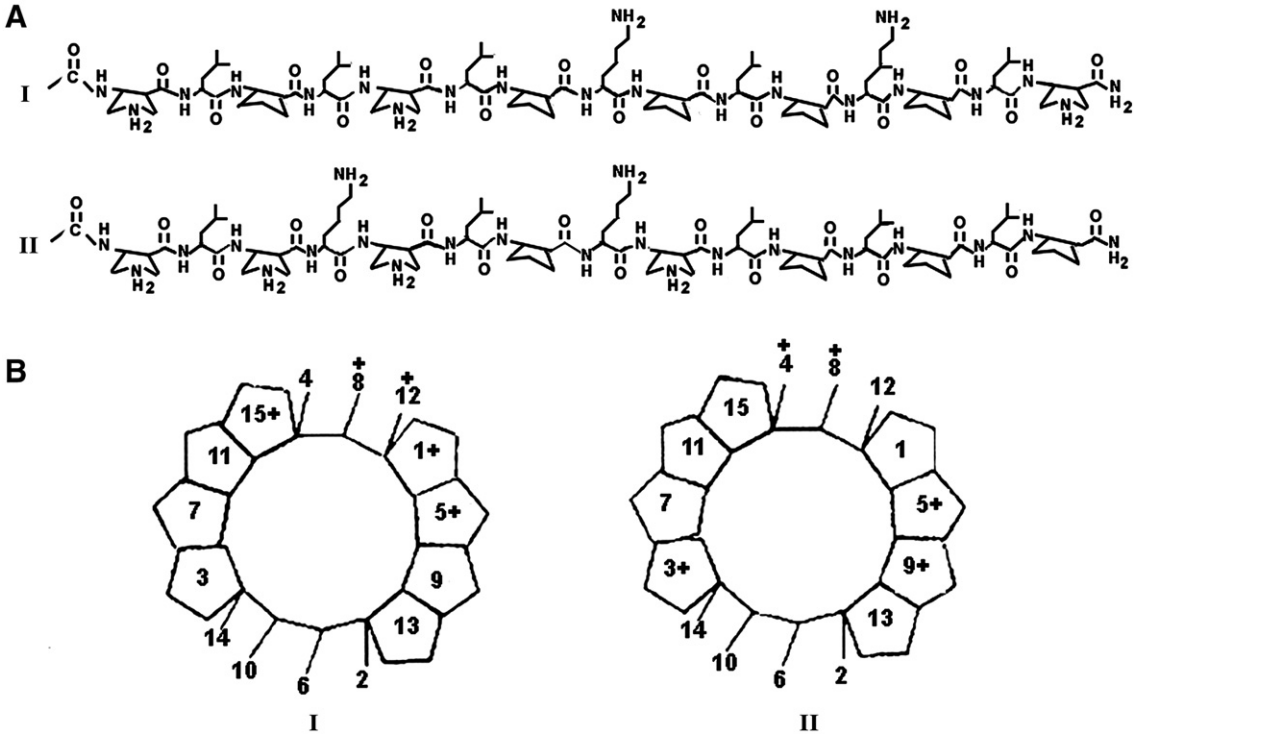

Fig. 2. (A) Structures of α/β-peptides I and II. (B) Schematic views of α/β-peptides I and II in idealized 14/15-helical conformations, viewed along the helix axis (these images are analogous to "helical wheel" diagrams for α-peptides). These perspectives show that when the α/β-peptides are helical, I is expected to be globally amphiphilic while II is not. Taken from [35].

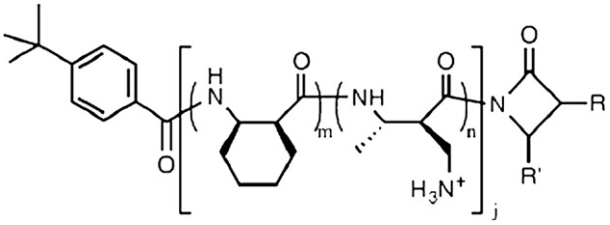

Fig. 3. We show the generic structure of these sequence-random copolymers. The version used in our studies has an average length of 21 subunits and an average molecular weight of 2800 (Mn/Mw=1.4) as well as *m*:*n*=2:3 (it is referred to as 3_{60} in [27]).

even in the presence of an anionic membrane surface. The peptide is unstructured in solution but forms aggregates containing antiparallel β-structure on an anionic membrane surface [31]. The Arg residues are involved in the binding of this peptide to anionic membrane surfaces [31]. Because the charges on the peptide are not spaced at alternate residues, we suggest that the β-structure formed on the membrane surface must have sufficient conformational flexibility to allow the guanidine groups of Arg to position themselves so as to interact with the negative charges of the membrane surface. It has been shown by ^{2}H NMR that this results in the formation of zones on the membrane with different rigidity and thickness [31]. This work also showed that the peptide did not cause destruction of the membrane and it was suggested that the presence of phase boundary defects allowed for increased membrane permeability [31].

OAKs are linear sequences of alternating acyl chains and cationic Lys residues (see Fig. 5) [36–39]. OAKs are rich in positive charge and because of the presence of the acyl groups, they are relatively hydrophobic and can partition well into membranes. These compounds do not form ordered secondary structures and are therefore capable of adapting their conformation to bind to anionic groups on a membrane surface and to induce lateral phase separation. We have shown both by DSC and by ^{31}P MAS/NMR that one of these OAKs, the octamer $C_{12}K\text{-}7\alpha_8$, is capable of interacting with anionic lipids and of segregating anionic and zwitterionic lipids [33].

4. Relationship of bacterial membrane lipid composition and the potency of certain antimicrobial agents

Bacteria vary widely in the lipid composition of their membranes and would therefore be expected to exhibit different sensitivities to antimicrobial compounds that act at the cell surface. With regard to agents that act by inducing lateral phase separation, one would expect that bacteria whose membranes are composed predominantly of anionic lipids would be more resistant to these antimicrobial agents since they would have insufficient zwitterionic phospholipids to form separate domains. However, these differences among bacterial species are relative and not absolute. The induction of lateral phase separation causing defects in membranes is just one of many mechanisms of action of antimicrobial agents. Thus, it is usually found that even bacteria that lack zwitterionic lipids are killed by agents that induce lateral phase separation, but probably by another mechanism. Generally PG, CL and PE account for a very large fraction of the phospholipids of bacterial membranes. However, other lipids are significant components of some bacteria. Such lipids can include glycolipids, lysyl-phosphatidylcholine and lysyl-cardiolipin. We

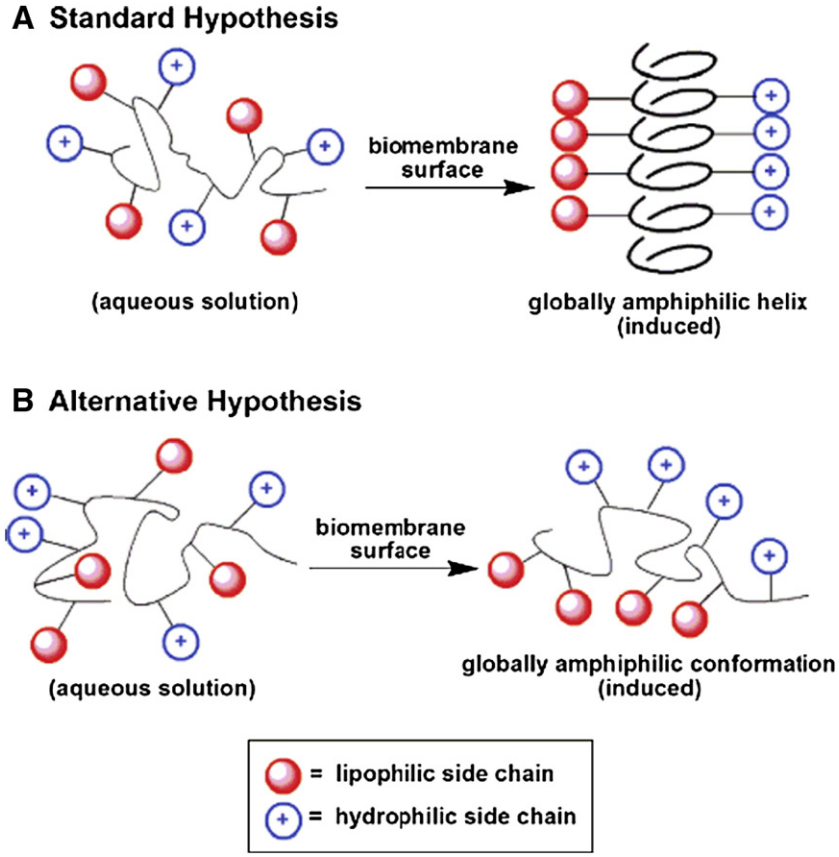

Fig. 4. Complementary hypotheses that can explain the antibacterial activity of host-defense peptides and synthetic oligomers and polymers that are designed to mimic these peptides. (A) The standard hypothesis for peptides such as magainins or cecropins involves induction of a globally amphiphilic helix folding pattern upon interaction with a bacterial membrane. The globally amphiphilic conformation is proposed to be responsible for disruption of the bacterial membrane. Variations on this hypothesis, all involving the induction of regular conformations, have been invoked to explain the activity of many unnatural antibacterial oligomers. (B) An alternative hypothesis, which can explain the activity reported here for random copolymers, involves induction of globally amphiphilic but irregular conformations in the presence of a bacterial membrane (taken from [27]).

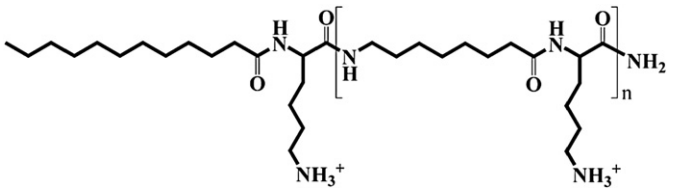

Fig. 5. Chemical structure of a typical OAK. Brackets define α8 (aminooctanoyllysyl) subunits where n=7 in the OAK, $C_{12}K\text{-}7_{\alpha 8}$, used to study phase segregation.

exclude from the discussions below analysis of the antimicrobial activity against *Enterococcus faecium*. This species contains 20% of the cationic lipid, lysyl-phosphatidylglycerol. This could both facilitate antimicrobial action by promoting phase segregation induced by a cationic antimicrobial agent, as well as inhibiting this action as a consequence of disfavouring sequestering these cationic agents to a more positively charged bacterial cell surface. There is also the presence of an outer membrane of Gram negative bacteria that can affect resistance, as well as the changes in the acyl chain composition and/or changes in lipid composition during the cell cycle. In addition, there is some limitation in the amount of information about bacterial membrane lipid compositions available in the literature. With some exceptions, many of the lipid analyses of bacterial membranes were performed using methodologies that are not as quantitative or comprehensive as those available today. In addition, the agents we have discussed are relatively new and in some cases there is not a great deal of literature available in which antimicrobial action was tested against a broad range of bacteria. Nevertheless, despite these limitations, we can demonstrate a strong correlation between the lipid composition of bacteria and the ability of antimicrobial agents to induce lateral phase separation.

In the case of α/β-peptide II, the minimal inhibitory concentration (MIC) against *E. coli* and against *B. subtilis* are both 6.3 μg/mL [34]. These bacterial species contain both anionic and zwitterionic lipids with 80 and 12%, respectively, of the zwitterionic phospholipid, PE. The α/β-peptide II can therefore segregate domains enriched in anionic or in zwitterionic lipid in these species. In contrast, *Staphylococcus aureus* has very little zwitterionic lipid and therefore should be resistant to α/β-peptide II because there are no other lipids for anionic ones to segregate from. In agreement with this, α/β-peptide II does have a higher MIC of 12.5 μg/mL against *S. aureus* [34], but the difference among these MICs is small and suggests that other mechanisms contribute to the antimicrobial action. However, the comparison between α/β-peptide II and its analog α/β-peptide I is consistent with the suggestion that with α/β-peptide II the additional mechanism is inducing lateral phase separation. The α/β-peptide I does not induce domain formation [29]. *E. coli* membranes contain both anionic and zwitterionic lipids and should thus be sensitive to agents that induce lateral phase separation. In agreement with the finding that α/β-peptide I does not induce phase separation while α/β-peptide II does, the MIC of α/β-peptide I against *E. coli* is 100 μg/mL, much greater that that for α/β-peptide II that is 6.3 μg/mL, indicating that the induction of lateral phase separation contributes to its toxicity.

In the case of the random cationic copolymer, the MIC against *S. aureus* that is devoid of zwitterionic lipid, is almost 10-fold higher than against *B. subtilis* that has 12% of the zwitterionic PE [35], And with the Gram negative bacteria, *E. coli*, it has a MIC that is two fold lower than that for *S. aureus* [35].

The MICs for cateslytin against various species of bacteria have been determined [40,41] and are summarized in Table 1, together with the lipid compositions of these bacteria. There are two groups of bacteria. One group comprising *Pseudomonas aeruginosa*, *E. coli* and *M. luteus* have MICs in the range 5–15 μM. All three of these bacterial

Table 1
Antimicrobial activity of human catestatin

Bacterial Species	% Total lipid				MIC (μM)
	CL	PG	PE	DMDG[a]	
Gram negative bacteria					
P. aeruginosa	8	3	60	–	6.2
E. coli	5	15	80	–	15
Gram positive bacteria					
M. luteus	5	60	–	30	5
Streptococcus (Group A)	50	50	–	–	75
S. aureus	42	58	–	–	>100

[a] DMDG is dimannosyldiacylglycerol.

species have membranes with both uncharged and anionic lipids that can phase-segregate. In the case of *M. luteus*, DMDG is an uncharged lipid. Bacteria largely devoid of uncharged lipids, *Streptococcus* and *S. aureus*, have much higher MICs, in the range of 75 to >100 μM. This would be consistent with the hypothesis that catestatin acts by clustering anionic lipids and forming phase boundary defects [31] and can do this only with bacteria that have substantial amounts of both cationic and uncharged lipid.

The antimicrobial activity of the OAK, $C_{12}K\text{-}7\alpha_8$, has been tested against many bacterial species (Table 2). One of the unusual features of the microbial specificity of this OAK is that it is generally more toxic to Gram negative bacteria than Gram positive ones. More commonly Gram negative bacteria are more resistant to antimicrobial agents because they have an additional protective barrier of the outer membrane. The outer membrane may also function to bind to a cationic antimicrobial agent and prevent its access to the cytoplasmic membrane. We believe Gram negative bacteria are more easily killed with OAKs because their membranes have a high concentration of the zwitterionic lipid PE together with anionic lipids. The OAK can therefore induce phase separation by preferentially interacting with and clustering the anionic lipid component. This is supported by the finding that two Gram positive bacteria, *Bacillus cereus* and *Bacillus polymyxa*, that have a high content of PE, also have a MIC that is considerably lower than it is for the two Gram positive bacteria whose membrane lipids are largely anionic (Table 2). An additional example is the Gram positive bacterial species *Listeria seeligeri* that has a MIC of 6.2 μM [39]. Two predominant lipids in this organism are cardiolipin and lysyl-cardiolipin [42]. Since lysyl-cardiolipin has equal numbers of positive and negative changes, *L. seeligeri*, is another example of a Gram positive bacteria that can form domains with anionic and uncharged lipids. Hence the MIC of *L. seeligeri* is much lower than that for bacteria whose membranes are composed largely of anionic lipids (Table 2).

The fact that the lipid composition of the bacteria will provide a predictable feature contributing to the sensitivity of the bacteria to certain antimicrobial agents can be used to design new drugs with a more limited range of bacterial toxicities. The mechanism of inducing

Table 2
Lipid composition and MIC of $C_{12}K\text{-}7_{\alpha 8}$ against various bacteria

Bacterial species	% Total lipid			MIC (μM)	Reference
	CL	PG	PE		
Gram negative bacteria					
E. coli	–	15	80	1.6–3.1	[39]
E. cloacae	3	21	74	1.6	[39]
Y. kristensenii	20	20	60	1.6	[39]
P. mirabilis	5	10	80	6.2	[39]
K. pneumoniae	6	5	82	3.1	[39]
P. aeruginosa	11	21	60	6.2	[39]
Gram positive bacteria					
Staphylococcus aureus	42	58	0	50	[39]
Streptococcus pneumonia	50	50	0	50	[33]
Bacillus cereus	17	40	43	12	[37,39]
Bacillus polymyxa	8	3	60	6.2	[33]

lateral phase separation will in general be more important for Gram negative bacteria than for Gram positive species since most Gram negative bacteria have significant amounts of both anionic and zwitterionic lipids. This may be an important feature in designing antimicrobial therapies, given that Gram negative bacteria are generally more resistant to antimicrobial agents.

5. Summary and conclusions

The chemical nature of antimicrobial agents is quite diverse. One would therefore not anticipate that they would all function by similar mechanisms. In this review we have focused on examples of antimicrobial agents that promote the formation of domains in membranes. These substances have multiple cationic charges arrayed in a flexible structure. In the examples we have chosen, the particular properties of the molecules endow them with an ability to segregate membrane domains. This combination of properties is somewhat specific since related analogs such as α/β-peptide I or the OAK, C_{12}K-5α_8, are much more weakly potent in inducing domain formation. Although this mechanism of domain formation is more important for some antimicrobial agents than for others, it likely contributes to some extent to the toxicity of many antimicrobial agents, most of which have many positive charges. Compounds that promote clustering of anionic lipids in model membranes are found to be selectively toxic to bacteria with both anionic and uncharged lipids in their membranes, indicating that a major contributing factor to the antimicrobial action of these agents is their ability to segregate lipids into domains. This can result in a lowering of the membrane permeability barrier as a consequence of the formation of packing irregularities at the locations where the two phases become juxtaposed. An attractive feature of this mechanism is that it can be used to rationally design antimicrobial agents with toxicity selective for certain organisms.

References

[1] K. Matsumoto, J. Kusaka, A. Nishibori, H. Hara, Lipid domains in bacterial membranes, Mol. Microbiol. 61 (2006) 1110–1117.

[2] J. deBony, A. Lopez, M. Gilleron, M. Welby, G. Laneelle, B. Rousseau, J.P. Beaucourt, J.F. Tocanne, Transverse and lateral distribution of phospholipids and glycolipids in the membrane of the bacterium *Micrococcus luteus*, Biochemistry 28 (1989) 3728–3737.

[3] M. Welby, Y. Poquet, J.F. Tocanne, The spatial distribution of phospholipids and glycolipids in the membrane of the bacterium *Micrococcus luteus* varies during the cell cycle, FEBS Lett. 384 (1996) 107–111.

[4] E. Mileykovskaya, Q. Sun, W. Margolin, W. Dowhan, Localization and function of early cell division proteins in filamentous *Escherichia coli* cells lacking phosphatidylethanolamine, J. Bacteriol. 180 (1998) 4252–4257.

[5] S. Vanounou, D. Pines, E. Pines, A.H. Parola, I. Fishov, Coexistence of domains with distinct order and polarity in fluid bacterial membranes, Photochem. Photobiol. 76 (2002) 1–11.

[6] S. Vanounou, A.H. Parola, I. Fishov, Phosphatidylethanolamine and phosphatidylglycerol are segregated into different domains in bacterial membrane. A study with pyrene-labelled phospholipids, Mol. Microbiol. 49 (2003) 1067–1079.

[7] H. Christensen, N.J. Garton, R.W. Horobin, D.E. Minnikin, M.R. Barer, Lipid domains of mycobacteria studied with fluorescent molecular probes, Mol. Microbiol. 31 (1999) 1561–1572.

[8] I. Fishov, C.L. Woldringh, Visualization of membrane domains in *Escherichia coli*, Mol. Microbiol. 32 (1999) 1166–1172.

[9] V. Norris, I. Fishov, Hypothesis: membrane domains and hyperstructures control bacterial division, Biochimie 83 (2001) 91–97.

[10] E. Mileykovskaya, W. Dowhan, Role of membrane lipids in bacterial division-site selection, Curr. Opin. Microbiol. 8 (2005) 135–142.

[11] A. Aranovich, G.Y. Gdalevsky, R. Cohen-Luria, I. Fishov, A.H. Parola, Membrane-catalyzed nucleotide exchange on DnaA. Effect of surface molecular crowding, J. Biol. Chem. 281 (2006) 12526–12534.

[12] A. Aranovich, A.H. Parola, I. Fishov, The reactivation of DnaA(L366K) requires less acidic phospholipids supporting their role in the initiation of chromosome replication in *Escherichia coli*, FEBS Lett. 581 (2007) 4439–4442.

[13] E. Mileykovskaya, W. Dowhan, R.L. Birke, D. Zheng, L. Lutterodt, T.H. Haines, Cardiolipin binds nonyl acridine orange by aggregating the dye at exposed hydrophobic domains on bilayer surfaces, FEBS Lett. 507 (2001) 187–190.

[14] E. Mileykovskaya, W. Dowhan, Visualization of phospholipid domains in *Escherichia coli* by using the cardiolipin-specific fluorescent dye 10-*N*-nonyl acridine orange, J. Bacteriol. 182 (2000) 1172–1175.

[15] W. Dowhan, E. Mileykovskaya, M. Bogdanov, Diversity and versatility of lipid–protein interactions revealed by molecular genetic approaches, Biochim. Biophys. Acta 1666 (2004) 19–39.

[16] F. Kawai, M. Shoda, R. Harashima, Y. Sadaie, H. Hara, K. Matsumoto, Cardiolipin domains in *Bacillus subtilis* marburg membranes, J. Bacteriol. 186 (2004) 1475–1483.

[17] C.M. Koppelman, B.T. Den, M.C. Duursma, R.M. Heeren, N. Nanninga, *Escherichia coli* minicell membranes are enriched in cardiolipin, J. Bacteriol. 183 (2001) 6144–6147.

[18] E. Mileykovskaya, Subcellular localization of *Escherichia coli* osmosensory transporter ProP: focus on cardiolipin membrane domains, Mol. Microbiol. 64 (2007) 1419–1422.

[19] T. Romantsov, S. Helbig, D.E. Culham, C. Gill, L. Stalker, J.M. Wood, Cardiolipin promotes polar localization of osmosensory transporter ProP in *Escherichia coli*, Mol. Microbiol. 64 (2007) 1455–1465.

[20] R. Mukhopadhyay, K.C. Huang, N.S. Wingreen, Lipid localization in bacterial cells through curvature-mediated microphase separation, Biophys. J. 95 (2008) 1034–1049.

[21] A. Nishibori, J. Kusaka, H. Hara, M. Umeda, K. Matsumoto, Phosphatidylethanolamine domains and localization of phospholipid synthases in *Bacillus subtilis* membranes, J. Bacteriol. 187 (2005) 2163–2174.

[22] R.M. Epand, H.J. Vogel, Diversity of antimicrobial peptides and their mechanisms of action, Biochim. Biophys. Acta 1462 (1999) 11–28.

[23] K.L. Brown, C. Cosseau, J.L. Gardy, R.E. Hancock, Complexities of targeting innate immunity to treat infection, Trends Immunol. 28 (2007) 260–266.

[24] Y. Shai, Mode of action of membrane active antimicrobial peptides, Biopolymers 66 (2002) 236–248.

[25] C. Marchand, K. Krajewski, H.F. Lee, S. Antony, A.A. Johnson, R. Amin, P. Roller, M. Kvaratskhelia, Y. Pommier, Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites, Nucleic Acids Res. 34 (2006) 5157–5165.

[26] E. Breukink, I. Wiedemann, C. van Kraaij, O.P. Kuipers, H.G. Sahl, B. de Kruijff, Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic, Science 286 (1999) 2361–2364.

[27] H.E. Hasper, N.E. Kramer, J.L. Smith, J.D. Hillman, C. Zachariah, O.P. Kuipers, K.B. De, E. Breukink, An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II, Science 313 (2006) 1636–1637.

[28] K. Matsuzaki, Magainins as paradigm for the mode of action of pore forming polypeptides, Biochim. Biophys. Acta 1376 (1998) 391–400.

[29] R.F. Epand, M.A. Schmitt, S.H. Gellman, R.M. Epand, Role of membrane lipids in the mechanism of bacterial species selective toxicity by two alpha/betaantimicrobial peptides, Biochim. Biophys. Acta 1758 (2006) 1343–1350.

[30] R.F. Epand, B.P. Mowery, S.E. Lee, S.S. Stahl, R.I. Lehrer, S.H. Gellman, R.M. Epand, Dual mechanism of bacterial lethality for a cationic sequence-random copolymer that mimics host-defense antimicrobial peptides, J. Mol. Biol. 379 (2008) 38–50.

[31] F. Jean-Francois, S. Castano, B. Desbat, B. Odaert, M. Roux, M.H. Metz-Boutigue, E.J. Dufourc, Aggregation of cateslytin beta-sheets on negatively charged lipids promotes rigid membrane domains. A new mode of action for antimicrobial peptides? Biochemistry 47 (2008) 6394–6402.

[32] L.M. Hays, J.H. Crowe, W. Wolkers, S. Rudenko, Factors affecting leakage of trapped solutes from phospholipid vesicles during thermotropic phase transitions, Cryobiology 42 (2001) 88–102.

[33] R.M. Epand, S. Rotem, A. Mor, B. Berno, and R.F. Epand, Bacterial membranes as a predictor of antimicrobial potency. J. Amer. Chem. Soc. (in press), doi:10.1021/ja8062327.

[34] M.A. Schmitt, B. Weisblum, S.H. Gellman, Unexpected relationships between structure and function in alpha,beta-peptides: antimicrobial foldamers with heterogeneous backbones, J. Am. Chem. Soc. 126 (2004) 6848–6849.

[35] B.P. Mowery, S.E. Lee, D.A. Kissounko, R.F. Epand, R.M. Epand, B. Weisblum, S.S. Stahl, S.H. Gellman, Mimicry of antimicrobial host-defense peptides by random copolymers, J. Am. Chem. Soc. 129 (2007) 15474–15476.

[36] I. Radzishevsky, M. Krugliak, H. Ginsburg, A. Mor, Antiplasmodial activity of lauryl-lysine oligomers, Antimicrob. Agents Chemother. 51 (2007) 1753–1759.

[37] I.S. Radzishevsky, S. Rotem, D. Bourdetsky, S. Navon-Venezia, Y. Carmeli, A. Mor, Improved antimicrobial peptides based on acyl-lysine oligomers, Nat. Biotechnol. 25 (2007) 657–659.

[38] I.S. Radzishevsky, T. Kovachi, Y. Porat, L. Ziserman, F. Zaknoon, D. Danino, A. Mor, Structure–activity relationships of antibacterial acyl-lysine oligomers, Chem. Biol. 15 (2008) 354–362.

[39] S. Rotem, I.S. Radzishevsky, D. Bourdetsky, S. Navon-Venezia, Y. Carmeli, A. Mor, Analogous oligo-acyl-lysines with distinct antibacterial mechanisms, FASEB J. 22 (2008) 2652–2661.

[40] J. Briolat, S.D. Wu, S.K. Mahata, B. Gonthier, D. Bagnard, S. Chasserot-Golaz, K.B. Helle, D. Aunis, M.H. Metz-Boutigue, New antimicrobial activity for the catecholamine release-inhibitory peptide from chromogranin A, Cell. Mol. Life Sci. 62 (2005) 377–385.

[41] K.A. Radek, B. Lopez-Garcia, M. Hupe, I.R. Niesman, P.M. Elias, L. Taupenot, S.K. Mahata, D.T. O'Connor, R.L. Gallo, The neuroendocrine peptide catestatin is a cutaneous antimicrobial and induced in the skin after injury, J. Invest. Dermatol. 128 (2008) 1525–1534.

[42] W. Fischer, K. Leopold, Polar lipids of four *Listeria* species containing L-lysylcardiolipin, a novel lipid structure, and other unique phospholipids, Int. J. Syst. Bacteriol. 49 (1999) 653–662.