Advances in Colloid and Interface Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

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

Historical perspective

The interaction of antimicrobial peptides with membranes

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ARTICLE INFO

Keywords:
Antimicrobial peptides
Arenicin
Phospholipids
Cd
IRRAS
Specular X-ray reflectivity

ABSTRACT

The interaction of antimicrobial peptides (AMPs) with biological membranes is in the focus of research since several years, and the most important features and modes of action of AMPs are described in this review. Different model systems can be used to understand such interactions on a molecular level. As a special example, we use 2D and 3D model membranes to investigate the interaction of the natural cyclic (Ar-1) and the synthetic linear molecule arenicin with selected amphiphiles and phospholipids. A panoply of sophisticated methods has been used to analyze these interactions on a molecular level. As a general trend, one observes that cationic antimicrobial peptides do not interact with cationic amphiphiles due to electrostatic repulsion, whereas with non-ionic amphiphiles, the peptide interacts only with aggregated systems and not with monomers. The interaction is weak (hydrophobic interaction) and requires an aggregated state with a large surface (cylindrical micelles). Anionic amphiphiles (as monomers or micelles) exhibit strong electrostatic interactions with the AMPs leading to changes in the peptide conformation.

Both types of peptides interact strongly with anionic phospholipid monolayers with a preference for fluid layers. The interaction with a zwitterionic layer is almost absent for the linear derivative but measurable for the cyclic arenicin Ar-1. This is in accordance with biological experiments showing that Ar-1 forms well defined stable pores in phospholipid and lipopolysaccharide (LPS) membranes (cytotoxicity). The synthetic linear arenicin, which is less cytotoxic, does not affect the mammalian lipids to such an extent. The interaction of arenicin with bacterial membrane lipids is dominated by hydrogen bonding together with electrostatic and hydrophobic interactions.

1. Introduction

Antimicrobial peptides (AMPs), also called host defense peptides [1], are an evolutionarily conserved component of the innate immune response system and are found in every organism [2–6]. They are essential components of the host defense against infections. Such peptides display remarkable activity against bacteria, fungi, viruses and parasites [7,8]. They are also involved in immunomodulatory activities and inflammatory processes [9–11]. Additionally, there is a certain degree of coupling between the innate and adaptive immune systems: antimicrobial peptides influence both, the quality and effectivity of immune and inflammatory responses [12].

The discovery of AMPs goes back to 1939 [13–15] when gramicidin was discovered. The first reported animal-originated AMP is defensin, which was isolated from rabbit leukocytes [16].

The target of AMPs is the membrane. AMPs are thought to overcome the resistance problem of traditional antibiotics ('the antibiotic crisis' [17]). It was argued that a resistance against AMPs could only be attained by a change of the lipid composition (different charge, changed fluidity). But this idea is too simple and unfortunately not true. Alterations of net surface charges, structural alterations in LPS, changes in the membrane proteins, increased production of proteolytic enzymes or glycocalyx shielding can inactivate AMPs [7,8,18]. A prospective resistance against AMPs in a broad medical use can never be excluded [19], but the structure-activity-relationship of natural AMPs should serve more as a template for the design of new peptides.

Besides that, antimicrobial peptides can also be involved in biochemical processes like the inactivation of nucleic acids and cytoplasmic proteins [18]. Many AMPs are active against cancer cells [5,20–23]. For example melittin (from the bee venom) inhibits tumor cell metastasis by reducing cell motility and migration [24]. The NK-2 (derivative from the porcine NK-lysin) killing activity correlates with the membrane exposure of negatively charged PS on the surface of cancer cells [21]. Magainin II (from frog skin) inhibited cell proliferation of bladder cancer cells in a dose-dependent manner [25], gomesin (from the spider *Acanthoscurria gomesiana*) significantly delayed subcutaneous murine melanoma development and increased the number of living treated animals with tumors below the allowed maximal size

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http://dx.doi.org/10.1016/j.cis.2017.06.001
Received 3 April 2017; Received in revised form 30 May 2017; Accepted 3 June 2017

limit [26].

It is still unclear why some AMPs kill cancer cells but others do not [27]. Beside the effect of opposite charges of the peptides and the membrane, differences in the fluidity and/or morphological changes of the membrane could be involved, as well as increased levels of sialic acid of glycolipids [20]. Obviously, the interplay between the peptide and membrane-based factors is of utmost importance [22], and this shall be explained in this review by means of some examples. We will show that physical-chemical studies of the membrane water interface can provide valuable information to understand and to control peptide function. We will do this by first describing the general questions and then concentrate on a few specific systems to demonstrate the power of recent methodical developments in interfacial science.

2. Structure-function relationships

2.1. Mode of action

The modes of action by which antimicrobial peptides kill bacteria include disrupting membranes, interfering with metabolism, and targeting cytoplasmic components.

> 5000 AMPs are identified and published in databases [28–30], but their mode of action is still not well understood [18,31,32]. As an example, the cationic, amphipathic, and alpha-helical peptides are typically 12–37 amino acid residues in length, and may have a kink or a central hinge region [8]. But their sequence and their activity differ markedly [28,33,34]. Since bacteria cells are mainly composed of negatively charged lipids, electrostatic interactions must be involved in the cell-disrupting mechanism. But an interaction based only on electrostatics is insufficient and ignores additional molecular mechanisms [35]. Due to the different composition of eukaryotic compared to vertebrate host cell membranes, most of the AMPs can distinguish between the target membranes [36], making them cell-selective. But also non-cell selectivity was observed for some AMPs, like melittin [37], LL-37 [38] and dermaseptin S4 [39].

Several modes of action are predicted for AMPs, including a 'carpet model', where the peptides interact primarily with the lipid head groups, or the formation of 'barrel-stave' or 'toroidal pores', where the peptides penetrate the lipid bilayer [11,28,34,40] (see Fig. 1). For the formation of pores, the peptide has to aggregate making this mode of action concentration dependent [41] or even target charge-dependent [40]. The disorganization of the membrane is accompanied by a change in the orientation of the peptide, as seen for cardiotoxins [42] and other AMPs, like protegrin, alamethicin, melittin, and magainin [43–45]. While for the formation of a 'toroidal pore' the peptides interact only with the head groups of the lipids, for the formation of a 'barrel-stave pore' the peptides permeate the bilayer.

For an interaction with the membrane via a 'carpet mechanism', the peptides cover the membrane and interact only with the lipid head groups. Therefore, the peptides are oriented parallel to the surface. The adaptation of the secondary structure and a penetration of the peptides into the hydrophobic core of the bilayer are not mandatory. The membrane is disrupted in a detergent-like way. Polymyxin B is thought to act via that mechanism [46]. It is discussed that the formation of toroidal pores is part of the carpet mechanism [31]. Magainin [41,44], alamethicin [47], melittin [44,47,50] and some channel peptides [48] can be bound in two states, and the formation of pores is transient [49,50], meaning a mixed mechanism in the peptide activity. The peptides can induce lesions by arranging parallel to the membrane, even without forming pores. But the exact mechanism is still unclear.

2.2. Structural properties

Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. AMPs are relatively small molecules. Their size varies from 6 amino acid residues for anionic peptides to > 59 amino acid residues for bactenecins. Even di- and tripeptides with antimicrobial activity have been reported.

2.2.1. Net charge

A positive charge is required for antimicrobial activity, therefore to the class of AMPs belong first of all the cationic peptides, which are rich in arginine or lysine amino acid residues, forming often highly defined cationic domains, or, in acidic environments, histidine. The net charge is ranging from + 2 to + 9. Small anionic AMPs which are rich in aspartic or glutamic acids act in complexes with Zn^{2+} (dermicidin from humans [51], maximin from amphibians [52], and other [53]).

There is a strong correlation between peptide cationicity and antimicrobial activity, as has been demonstrated in a number of studies [54,55]. Increasing charge results in increasing antibacterial activities against Gram-negative and Gram-positive pathogens [56]. However, there is a limit beyond which increasing positive charge no longer causes increased activity. Too high net charge leads to an increased hemolytic propensity and a loss of antimicrobial activity [56,57].

2.2.2. Sequence and specific amino acid residues

Peptides often contain basic amino acid residues as lysine or arginine, the hydrophobic residues alanine, leucine, phenylalanine or tryptophan, and other residues such as isoleucine, tyrosine and valine. The ratio of hydrophobic to charged residues can vary from 1:1 to 2:1.

It has been suggested that the polar character of the tryptophan amide group and the tyrosine hydroxyl, along with their hydrophobic ring structures, favour their localization at the polar/apolar interface. Lysine and arginine are often at the lipid/water interface, with the positively charged groups at the ends of their aliphatic side chains extending toward the polar membrane surface. Statistical analyses reveal that the frequently used amino acid residues (> 10%) are Ala and Gly in bacterial peptides, Cys and Gly in plant peptides, Ala, Gly and Lys in insect peptides, and Leu, Ala, Gly and Lys in amphibian peptides [58].

2.2.3. Conformation

The secondary structures of AMPs follow 4 themes, including α-helical (Fig. 2 middle), β-stranded (Fig. 2 right) due to the presence of 2 or more disulfide bonds, β-hairpin or loop due to the presence of a single disulfide bond and/or cyclization of the peptide chain, and nearly linear extension (Fig. 2 left).

Many AMPs are believed to exist in relatively unstructured or extended conformations prior to interaction with target cells. Others are organized in specific conformations by intramolecular bonds. Upon binding to pathogen membranes, peptides may undergo significant conformational changes to helical or other structures that affect antimicrobial activity. Although antimicrobial peptides differ widely in sequence and source, several motives appear predominant in their three-dimensional topology, and peptides have been categorized accordingly. The two largest groups are the α-helical and β-sheet peptides. The α-helical antimicrobial peptides are abundant in the extracellular fluids of insects and frogs. Many of these peptides exist as extended or unstructured conformers in solution but become helical upon interaction with amphipathic phospholipid membranes. This change in conformation would also likely alter the peptide hydrophobic moment. Another potentially important aspect of the conformational phase transition is, that it may prevent indiscriminant membranolytic activity until the peptide identifies an appropriate target surface. Thus, a lack of bioactive structure at non-target sites may be an important means by which antimicrobial peptides minimize host cell toxicity. The best studied representative of α-helical peptides is magainin [59].

The β-sheet peptides represent a highly diverse group of molecules. In comparison with helix-forming peptides, which are usually disordered in aqueous environment, β-sheet antimicrobial peptides are typically much more ordered in aqueous solution and in membrane environments, due to constraints imposed by disulfide bonds or