Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Proline-15 creates an amphipathic wedge in maculatin 1.1 peptides that drives lipid membrane disruption

Marc-Antoine Sani [a,1], Tzong-Hsien Lee [b,1], Marie-Isabel Aguilar [b,*], Frances Separovic [a,*]

[a] School of Chemistry, Bio21 Institute, University of Melbourne, VIC 3010, Australia
[b] Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC 3800, Australia

ARTICLE INFO

Article history:
Received 24 April 2015
Received in revised form 5 June 2015
Accepted 11 June 2015
Available online xxxx

Keywords:
Antimicrobial peptide
Maculatin 1.1
Model membranes
Peptide–lipid interaction
Dual polarization interferometry
Dye leakage

ABSTRACT

The membrane interaction of peptides derived from maculatin 1.1 and caerin 1.1, with the sequence motif of N and C termini of maculatin 1.1, was compared in order to understand the role of these common sequence motifs, which encompass critical proline residues, on peptide secondary structure and on membrane binding and disruption in zwitterionic and anionic membranes. The peptides incorporated a single substitution with lysine or deletion of the central region to mimic the length of the antimicrobial peptides, citropin 1.1 and aurein 1.2. The impact of these changes in the sequence, length and physicochemical properties, on lytic activity and structure was assessed by dye-release from lipid vesicles and the change in the bilayer order as a function of membrane-bound peptide mass. All peptides adopted similar degrees of helical structure in both membrane systems. In addition, all peptide analogues were less active than either maculatin 1.1 or caerin 1.1 in dye release assays. The membrane binding was analyzed by dual polarization interferometry and the results showed that membrane binding was significantly affected by changes in the hydrophobic environment of Pro-15. Moreover, changes in the relative distribution of charge and hydrophobicity flanking Pro-15 also caused significant changes to the membrane order. Overall, the proline residue plays an important role in inducing a peptide structure that enhances the activity of these antimicrobial peptides.

1. Introduction

The alarming increase in antibiotic resistance presents a new challenge for medicinal chemistry to find potent alternatives that also limit natural selection of resistant strains. Among the innate immune system of higher organisms is found a plethora of relatively short (<50 amino acids) cationic peptides that have attracted significant interest. Their bactericidal mechanism is promising since their mode of action is to compromise the integrity of lipid membranes, which thereby avoids the activation of intracellular defense mechanisms [1,2].

First discovered in the late thirties, antimicrobial peptides (AMPs) are usually short cationic peptides, mainly adopting a helical structure, that induce lipid membrane leakage, either by forming a pore or by removing lipids in a detergent-like manner [3]. Many studies aim to identify the effect of amino acids in the primary sequence, mutating native AMP or trying to reproduce a certain scaffold with synthetic sequences. The main features that have been identified as important for activity are: amphipathicity that is promoted by the distribution of polar versus hydrophobic amino acids along the long axis of the structured peptide, the net charge and the length of the peptide. Peptides shorter than 15 residues generally act in a detergent-like manner while longer peptides may insert and span the hydrophobic core of the lipid membrane to form pores. Overall, a certain threshold of peptide concentration must be reached before membrane disruption. Yet, despite more than two thousand AMPs having been identified [4], the relationship between potency and AMP sequence is still elusive. This is partly due to the lack of information on the changes in the membrane structure that accompany the binding of AMPs to the target membrane.

We have selected two natural AMPs secreted on the skin of Australian tree frogs, maculatin 1.1 (Mac-1) and caerin 1.1 (Cae-1) as starting scaffolds to examine the structure–activity relationship of these two similar peptides. Maculatin 1.1 contains 21 residues and a proline residue which has been shown to play a critical role in the disruption of the bilayer [5,6], while caerin 1.1 contains 25 amino acids and two proline residues. Eight peptides were synthesized based on these two cationic peptides in which amino acid residues were either substituted or deleted (Table 1) in the region surrounding the proline residues. In the first category, an additional charge was added to Mac-1 by substituting Lys at residues 11 and 12. In the second category, either one or two turns of an α-helix were removed. The binding of these AMPs with two lipid membrane models was analyzed: the neutral or zwitterionic system of the major phospholipid found in eukaryotic cell membranes, palmitoyloleoylphosphatidylcholine (POPC); and a negatively charged

* Corresponding authors.
E-mail addresses: mibel.aguilar@monash.edu (M.-I. Aguilar), fs@unimelb.edu.au (F. Separovic).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.bbamem.2015.06.013
0005-2736/

Table 1
Physicochemical properties of Mac-1 and analogues.

Sequence	Short name	No. of residues	M_W (M + H) Da	Charge (pH 7)	Hydrophobicity[a]
Maculatin — charge density changes					
GLFGVLAKVAAHVVPAIAEH F-NH_2	Mac-1	21	2145.77	+1	1.3
GLFGVLAKVAA**K**VVPAIAEHF-NH_2	Mac-2	21	2136.44	+2	1.3
GLFGVLAKVA**K**HVVPAIAEHF-NH_2	Mac-3	21	2202.42	+2	1.0
Maculatin → Citropin → Aurein					
GLFGVLAK HVVPAIAEHF-NH_2	Mac-4	18	1904.14	+1	1.1
GLFGVLAKVAA AIAEHF-NH_2	Mac-5	17	1712.10	+1	1.4
GLFDVIKK VASVIGGL-NH_2	citropin	16	1614.98	+1	1.3
GLFGVLAK IAEHF-NH_2	Mac-6	13	1400.10	+1	1.1
GLFDIIKK IAESF-NH_2	aurein	13	1480.79	+1	1.1
Caerin → Maculatin					
GLLSVLGSVAKHVLPHVVPVIAEHL-NH_2	Cae-1	25	2583.77	+1	1.2
GLLSVLGSVAKHV VPVIAEHL-NH_2	Cae-2	21	2137.42	+1	1.3

[a] Kyte and Doolittle hydrophobicity scale [23].

system of POPC with 30% palmitoyloleoylphosphatidylglycerol (POPG). The change in secondary structure and adsorption on and disruption of lipid bilayers was then investigated in terms of the peptide physicochemical properties versus the membrane surface charge. On the whole, the results revealed that the topological distribution of amino acid residues around the critical proline residue (Fig. 1) strongly influences the membrane binding and disruption by Mac-1.

2. Material and methods

2.1. Materials

All peptides were obtained from the Bio21 peptide synthesis facility (Melbourne, Australia) with a >95% purity. The peptides were washed in 5 mM HCl solution and lyophilised overnight to remove residual trifluoroacetic acid as described [7]. Palmitoyloleoylphosphatidylcholine (POPC) and palmitoyloleoylphosphatidylglycerol (POPG) phospholipids were purchased from Avanti Polar Lipids (Alabaster, USA) and were used without further purification. Calcein, Aprotonin (A3886), Triton-x100 and Sephadex G-100 gel filtration media were purchased from Sigma (St Louis, USA).

2.2. CD sample preparation

Peptides were dissolved in 10 mM phosphate and 1 mM buffer solution (pH 7.4), except Mac-5 which showed higher solubility in Milli-Q water. For all peptides, stock solutions containing 1 mg/mL were made. The stock solution was sonicated (10 s) and vortexed prior to each use. To prepare binary vesicles, lipids were co-solubilized in chloroform/methanol (3:1, v/v) before removal of solvents by rotary evaporation. Lipids were hydrated in Milli-Q water and lyophilised overnight. The resultant lipid powders were re-suspended in 10 mM phosphate and 1 mM NaCl buffer solution (pH 7.4). The homogeneous solutions were then extruded 10 times through an Avanti Mini-Extruder (Alabaster, USA) using polycarbonate filters to produce LUV of 200 nm diameter. The size of the LUV was confirmed by dynamic light scattering (DLS) performed on a Nano Zetasizer (Malvern Instruments Ltd, UK). Appropriate volumes of peptide stock solution and lipid vesicle dispersion were mixed to produce 160 μL samples with a fixed peptide concentration of 100 μM and 3 mM lipid.

2.3. CD measurements

CD spectra were acquired on a Chirascan spectropolarimeter (Applied Photophysics Ltd, UK) between 180 and 260 nm using a 0.1 mm pathlength cylindrical quartz cell (Starna, Hainault, UK). Spectra were acquired with 1 nm data intervals, 1 s integration time and 2 scans accumulation. Signal was recorded as milli-degrees at 25 °C.

2.4. CD spectral deconvolution

Spectra were zeroed at 260 nm and normalized to give units of mean-residue ellipticity (MRE) according to $[\theta]_{MRE} = \theta/(c \times l \times N_r)$, where θ is the recorded ellipticity in milli-degrees, c is the peptide concentration in dmol · L^{-1}, l is the cell path-length in cm, and N_r is the number of residues per peptide. The helicity was calculated from the Luo–Baldwin formula $H\alpha\ (\%) = (\theta_{222nm} - \theta_C)/(\theta^{\infty}_{222nm} - \theta_C)$. with $\theta_C = 2200 - 53\ T$, $\theta^{\infty}_{222nm} = (-44{,}000 + 250\ T)(1\text{-}k/N_{Residues})$, and $k = 4$ as described for unrestricted peptides [8,9].

2.5. Calcein solution preparation

Calcein was initially insoluble in water and was, therefore, dissolved in 4 eq. NaOH and vortexed until completely dissolved. Appropriate volumes of Tris and NaCl were added to yield an 80 mM calcein stock in 30 mM Tris, 20 mM NaCl before final adjustment to pH 7.3 with HCl.

2.6. Calcein encapsulation in large unilamellar vesicles

To prepare calcein-loaded large unilamellar vesicles (LUVs), lipids were suspended in 250 μL of a dye solution made of 80 mM calcein, 20 mM NaCl and Tris 30 mM (pH 7.3). The solutions were freeze/thawed five times and then extruded 10 times through an Avanti Mini-Extruder (Alabaster, USA) using 0.2 μm polycarbonate filters to produce LUVs of a theoretical 200 nm diameter. Samples were extruded above the corresponding main gel-to-fluid chain melting temperatures of the used lipids. Separation from free dye was obtained by gel filtration using Sephadex G-100 column media. The gel media and running buffer were carefully degassed before use to prevent air bubbles forming in the column. Samples were eluted under gravity with 30 mM Tris/100 mM NaCl (pH 7.3) running buffer solution at ~1 mL/min. Approximately 1 mL fractions were collected, with the two most concentrated fractions pooled prior to lipid concentration determination. Phospholipid concentrations were determined in triplicate using the phosphorus assay of Anderson et al. [10]. In parallel, lipids were also suspended in a calcein-free buffer to produce similar LUV solutions. The diameter of the calcein-free LUV was determined by DLS measurements.

Please cite this article as: M.-A. Sani, et al., Proline-15 creates an amphipathic wedge in maculatin 1.1 peptides that drives lipid membrane disruption, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamem.2015.06.013