Biochimica et Biophysica Acta 1788 (2009) 1537–1550

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

The dermaseptin superfamily: A gene-based combinatorial library of antimicrobial peptides

Pierre Nicolas *, Chahrazade El Amri

Equipe peptidome de la Peau des Amphibiens, FRE 2852 Université Pierre et Marie Curie – Paris 6, Tour 43, 2, Place Jussieu 75252 Paris cedex 05, France

ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 14 September 2008
Accepted 18 September 2008
Available online 26 September 2008

Keywords:
Dermaseptin
Frog skin
Diversifying selection
Antimicrobial peptide
Hylidae
Amyloid

ABSTRACT

Skin secretions of hylid frogs show amazing levels of interspecific and intraspecific diversity and are comprised of a cocktail of genetically-related, but markedly diverse antimicrobial peptides that are grouped into a superfamily, termed the dermaseptins, comprising several families: dermaseptins (sensu stricto), phylloseptins, plasticins, dermatoxins, phylloxins, hyposins, caerins, and aureins. Dermaseptin gene superfamily evolution is characterized by repeated gene duplications and focal hypermutations of the mature peptide coding sequence, followed by positive (diversifying) selection. We review here molecular mechanisms leading to these vast combinatorial peptide libraries, and structural and functional properties of antimicrobial peptides of the dermaseptin and plasticin families, as well as those of dermaseptin S9, an amyloidogenic peptide with antimicrobial and chemoattractant activities.

Contents

Abbreviations: ATR-FTIR, Attenuated total reflectance Fourier transform infrared; CD, circular dichroism; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine; DRP, dermaseptin-related peptide; DRS or DS, dermaseptin; DD, dermadistinctin; DRG, dermaseptin-gene related peptide; DRT, dermatoxin; ERK, extracellular signal-regulated kinase; FPRL-1, formyl peptide receptor Like 1; fMLP, formyl methionyl-leucyl-phenylalanine; FTIR, Fourier transform infrared; HPS, hyposin; IRP, insulin-related-peptide; MIC, minimal inhibitory concentration; NMR, nuclear magnetic resonance; ONPG, *o*-nitrophenyl β-D galactopyranoside; PLS, phylloseptin; PBN, *Phyllomedusa bicolor* new; PLX, phylloxin; PBS, phosphate-buffered saline; PTX, Pertussis toxin; SPR, surface plasmon resonance; SDS, sodium dodecyl sulfate; TFE, trifluoroethanol

* Corresponding author. Tel.: +33 1 44 27 95 36; fax: +33 144 27 59 94.
E-mail address: pierre.nicolas@upmc.fr (P. Nicolas).

0005-2736/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2008.09.006

1. Introduction

The dermaseptins are a superfamily of antimicrobial peptides that are made in the skin of Hylidae and Ranidae frogs. These peptides are genetically related, with a remarkable identity in signal sequences and intervening sequences of their preproforms but have markedly diverged to yield several families of peptides that are structurally and functionally distinct. Different species of frogs are equipped with different sets of peptides belonging to different families. The impressive divergence between and within frog species is such that no single peptide from one species has been found with an identical amino acid sequence in another; orthologous peptides, as well as paralogous peptides, have different amino acid sequences in different species, and often display differential biological activity. The studies on the evolution and diversity of frog skin antimicrobial peptides may help reveal new peptides targeting specific microorganisms against which few therapeutic armaments are available. In addition, the discovery of new isoforms with novel structural and biochemical properties may also shed light on the exact roles of various parameters, such as net charge, percent of helical/β-structure, amphipathy and conformational flexibility, on the ability of antimicrobial peptides to bind to and disrupt bacterial membranes.

2. The dermaseptin superfamily: structurally and functionally disparate peptides originating from a single gene family

Amphibian skin is an important source of gene-encoded antimicrobial peptides, with more than half of the ~900 eukaryotic peptides described to date isolated from South American Hylidae or European, Asian or North American Ranidae [http://www.bbcm.univ.trieste.it.]. Hylids (treefrogs) are one of the largest families of frogs. There are about 51 genera that are arranged in three subfamilies: *Pelodryadinae*, *Phyllomedusinae*, and *Hylinae*. The *Phyllomedusinae* subfamily comprised 57 species divided into seven genus: *Agalychnis*, *Cruziohyla*, *Hylomantis*, *Pachymedusa*, *Phasmahyla*, *Phrynomedusa* and *Phyllomedusa* (Table 1). More than 80 antimicrobial peptides have been isolated from only twelve species of the *Phyllomedusinae* subfamily, belonging to the genera *Agalychnis*, *Hylomantis, Pachymedusa,* and *Phyllomedusa*. Multiple alignment analysis clearly showed that these peptides can be grouped to form 7 different families [1]. To date these families include[1] (1) the dermaseptins (sensu stricto) from *Phyllomedusa sauvagii, P. bicolor, P. hypochondryalis, P. distincta, P. oreades, P. tarsius, P. trinitatis, Pachymedusa dacnicolor, Agalichnis annae, A. callidryas, A. litodryas,* and *Hylomantis lemur* (Table 1). These peptides share a signature pattern consisting of a conserved Trp residue at position 3 and an AA(A/G)KAAL(G/N)A consensus motif in the midregion; (2) the phylloseptins from *Phyllomedusa bicolor, P. hypochondryalis, P. oreades,* and *Hylomantis lemur* (Table 2); (3) the plasticins from *Phyllomedusa sauvagii, P. bicolor, Pachymedusa dacnicolor*, and *Agalichnis annae,* which are rich in Gly and Leu residues arranged in regular 5-mer motifs GXXXG (where X is any amino acid residue) (Table 2); (4) the dermatoxins from *Phyllomedusa sauvagii, P. bicolor, Pachymedusa dacnicolor*, and *Agalichnis annae* (Table 2); (5) the phylloxins from *Phyllomedusa sauvagii* and *P. bicolor* (Table 2); (6) the hypoxins from *Phyllomedusa hypochondryalis* (Table 2), and (7) the orphan peptides from *Phyllomedusa sauvagii, Pachymedusa dacnicolor,* and *Agalichnis annae,* which do not resemble any members of the other peptide families (Table 2). All these peptide families show such a considerable structural variety that their evolutionary relationships would never have been apparent without the strong conservation of their biosynthetic precursor preproregions. This conserved preproregion is the hallmark of the dermaseptin superfamily (see Section 3).

Australian frogs belonging to the *Pelodryadinae* subfamily of the family Hylidae also produce in their skin numerous antimicrobial peptides (caerins, aureins, maculatins..) that have no structural similarity with South American hylid peptides, but the signal peptide and acidic propiece of the precursors of the aureins and caerins are highly similar to the corresponding regions of the precursors of the South American hylid antimicrobial peptides (Table 3). Similarly, numerous disulfide-containing antimicrobial peptides from Eurasian and North American ranid frogs that belong to the brevinins, esculentins, nigrocins, palustrins, ranacyclin, ranalexin, ranatuerins, and temporin families have precursors whose preproregions strikingly resemble those of preprodermaseptins [2]. Frog skin secretions are also a rich source of biologically active neuropeptides and hormones that are very similar to mammalian peptides that are produced in the brain and the gastrointestinal tract. Most interesting, preprodermaseptins-encoding peptides also include the D-amino acid containing opioid peptides, dermorphins and deltorphins from *P. sauvagei* and *P. bicolor*, respectively, the bradykinin-related peptide [Thr^6]-phyllokinin from *P. sauvagei*, as well as tryptophyllin-1, a myoactive peptide from *Pachymedusa dacnicolor* relaxing mammalian arterial smooth muscles and contracting small intestinal smooth muscles [3–9]. The discovery of the considerable extent of sequence identity between the neuropeptide and antimicrobial peptide precursors is unprecedented. Thus, although the two groups of peptides are genetically related and belong to the same gene superfamily, they have strongly diverged to yield families of peptides that are both structurally and functionally distinct.

3. The dermaseptin superfamily: gene duplication, focal hypermutation, and diversifying selection generated a gene-based combinatorial peptide library

3.1. The one gene-one sequence paradigm

Peptides of the dermaseptin superfamily are synthesized in the multi-nucleated cells of the granular glands of the skin as prepropeptides that are processed by the removal of the signal peptide to the proform and then stored in the large granules of the glands. Glands may release their content onto the skin surface by a holocrine mechanism involving rupture of the plasma membrane and extrusion of the granules through a duct opening to the surface. Each precursor results from transcription of a single genetic locus that encodes a single mature peptide. This one gene-one sequence paradigm contrasts with the strategy that is employed by vertebrates to produce families of neuropeptides [10]. The canonical precursor architecture

[1] The proposed nomenclature is as follows [1]: the name of the first peptide (parent peptide) that has been isolated, or that will be isolated from a *Phyllomedusinae* frog species should be used as a headgroup name of each peptide belonging to the considered family. Orthologous peptides should bear the name of the parent peptide, followed by the first capital letter of the name of the *Phyllomedusinae* species from which they have been isolated. Paralogous peptides should bear the name of the parent peptide, followed by the first capital letter of the name of the *Phyllomedusinae* species from which they have been isolated, and by numbers. In some cases, classification of antimicrobial peptides using the parent peptide's name followed by one capital letter is insufficient as several species of the *Phyllomedusinae* sub-family share the same first letter. In these cases, the second letter written in its small form should be added to the first letter of the name followed by numbers for the paralogous peptides.

Table 1
The Dermaseptin family

Original name	Sequence	New name	Abbreviation
DRS B1 [81, 82]	AMWKDVLKKIGTVALH-AGKAALGAV-ADTISQ-----	Dermaseptin-B1	DRS-B1
DRS B2 [22, 82]	GLWS-KIKEVGKEAAKAAAKAA-GKAALGAVSEAV---	Dermaseptin-B2	DRS-B2
DRS B3 [82]	ALWKNMLKGIGKL----AGQAALGAVKTL-VGAE----	Dermaseptin-B3	DRS-B3
DRS B4 [82]	ALWKDILKNVGK----AAGKAVLNTV-TDMVNQ-----	Dermaseptin-B4	DRS-B4
DRS B5 [82]	GLWN-KIKEAAKS----AGKAALGFVN-EMV-------	Dermaseptin-B5	DRS-B5
DRS B6 [82]	ALWKDILKN--------AGKAALNEINQL-VNQ-----	Dermaseptin-B6	DRS-B6
DRS DRG1 [11]	GLWS-NIKTAGKE----AAKAALKAAGKAALGAVTDAV	Dermaseptin-B7	DRS-B7
DRS DRG2 [11]	GLWS-KIKEAGKAVLTAAGKAALGA--------VSDAV	Dermaseptin-B8	DRS-B6
DRS DRG3 [12]	ALWKTIIKGAGKMIGSLA-KNLLGSQAQPES-------	Dermaseptin-B9	DRS-B9
DRS S1 [21]	ALWKTMLKKLGTMALH-AGKAALGAA-ADTISQGTQ--	Dermaseptin-S1	DRS-S1
DRS S2 [20]	ALWFTMLKKLGTMALH-AGKAALGAA-ANTISQGTQ--	Dermaseptin-S2	DRS-S2
DRS S3 [20]	ALWKNMLKGIGKL----AGKAALGAVKKL-VGAES---	Dermaseptin-S3	DRS-S3
DRS S4 [20]	ALWMTLLKKVLK----AAAKA-LNAVLVGANA------	Dermaseptin-S4	DRS-S4
DRS S5 [20]	GLWS-KIKTAGKSV-----KAAA----KAAVKAVTNAV	Dermaseptin-S5	DRS-S5
DRS S6 [83]	GLWS-KIKTAGKE----AAKAAAKAAGKAALNAVSEAI	Dermaseptin-S6	DRS-S6
DRS S7 [83]	GLWKSLLKNVGK----AAGKAALNAV-TDMVNQ-----	Dermaseptin-S7	DRS-S7
DRS S8 [83]	ALWKTMLKKLGTVALH-AGKAALGAA-ADTISQ-----	Dermaseptin-S8	DRS-S8
DRS S11 [67]	ALWKTLLKGAGKVFGHVA-KQFLGSQGQPES-------	Dermaseptin-S11	DRS-S11
DRS S12 [67]	GLWS-KIKEAAKT----AGKMAMGFVN-DMV-------	Dermaseptin-S12	DRS-S12
DRS S13 [67]	GLRS-KIKEAAKT----AGKMALGFVN-DMA-------	Dermaseptin-S13	DRS-S13
DRP-AA-3-1 [84]	SLWS-KIKEMAAT----AGKAALNAVTGM-VNQ-----	Dermaseptin-A3	DRS-A3
DRP-AA-3-3 [84]	GMFTNMLKGIGKL----AGQAALGAVKTLA--------	Dermaseptin-A4	DRS-A4
DRP-AA-3-6 [84]	GMWS-TIRNVGKS---AA-KAANLPAKAA-LGAISEAV	Dermaseptin-A5	DRS-A5
DRP-PD-2-2 [84]	ALWKTLLKKVGKV----AGKAVLNAV-TNMANQNEQ--	Dermaseptin-DA2	DRS-DA2
DRP-PD-3-3 [84]	GMWS-KIKNAGKA---AA-KASKKAAGKAALGAVSEAL	Dermaseptin-DA3	DRS-DA3
DRP-AC-3 [6]	SVLS-TITDMAK----AAGRAALNAITGL-VNQ-----	Dermaseptin-C3	DRS-C3
PEAK-2.9 [85]	AVWKDFLKNIGK----AAGKAVLNSV-TDMVNE-----	Dermaseptin-LI1	DRS-LI1
DRS-H1 [86]	GLWKSLLKNVGV----AAGKAALNAV-TDMVNQ-----	Dermaseptin-H1*	DRS-H1
DRS-H2 [86]	ALWKSLLKNVGV----AAGKAALNAV-TDMVNQ-----	Dermaseptin-H2	DRS-H2
DRS-H3 [52]	GLWS-TIKNVA—A---AAGKAALGAL----------	Dermaseptin-H10*	DRS-H10
DRS-H4 [52]	GLWS-TIKNVGKEAAIAAGKAALGAL----------	Dermaseptin-H12*	DRS-H12
DRS-H5 [52]	GLWS-TIKNVGKEAAIAAGKAVLGSL----------	Dermaseptin-H13*	DRS-H13
DRS-H6 [52]	GLWS-TIKQKGKEAAIAAAKAA-GQAALGAL-------	Dermaseptin-H14*	DRS-H14
DRS-H7 [52]	GLWS-KIKDVA-A---AAGKAALG-AVNEAL-------	Dermaseptin-H15*	DRS-H15
DSHYPO01 [87]	GLWS-TIKNVGKEAAIAAGKAALGAL----------	Dermaseptin-H3*	DRS-H3
DSHYPO02 [88]	GLWKSLLKNVGV----AAGKAALNAV-TDMVNQ-----	Dermaseptin-H4*	DRS-H4
DSHYPO03 [88]	ALWKDVLKKIGTVALH-AGKAAFGAA-ADTISQGGS--	Dermaseptin-H5*	DRS-H5
DSHYPO04 [88]	GLWS-TIKQKGKEAAIAAAKAA-GKAVLNAASEAL---	Dermaseptin-H6*	DRS-H6
DSHYPO05 [88]	GLWS-TIKQKGKEAAIAAAKAA-GQAALGAL-------	Dermaseptin-H7*	DRS-H7
DSHYPO06 [88]	GLWS-TIKQKGKEAAIAAAKAA-GQAVLNSASEAL---	Dermaseptin-H8*	DRS-H8
DSHYPO07 [88]	GLWS-TIKQKGKEAAIAAAKAA-GQAALNAASEAL---	Dermaseptin-H9*	DRS-H9
DDM [89]	ALWKTMLKKLGTMALH-AGKAAFGAA-ADTISQ-----	Dermaseptin-DI3	DRS-DI3
DDK [89]	GLWS-KIKAAGKEAAKAAAKAAGKAA----LNAVSEAV	Dermaseptin-DI1	DRS-DI1
DDL [89]	ALWKTLLKNVGK----AAGKAALNAV-TDMVNQ-----	Dermaseptin-DI2	DRS-DI2
DDQ1 [89]	GLWS-KIKEAAKT----AGLMAMGFVN-DMV-------	Dermaseptin-DI5	DRS-DI5
IRP [32]	ALWKDILKNVGK----AAGKAVLNTV-TDMVNQ-----	Dermaseptin-TR1	DRS-TR1
DRS-L1 [90]	GLWS-KIKEAAK----AAGKAALNAVTGL-VNQGDQPS	Dermaseptin-L1	DRS-L1
DS-01 [29]	GLWS-TIKQKGKEAAIAAAKAA-GQAALGAL-------	Dermaseptin-O1	DRS-O1
DRS-LIKE [91]	ALWKDVLKKIGTVALH-AGKAALGAV-ADTISQ-----	Dermaseptin-TA1	DRS-TA1

Amino acid sequences of dermaseptins from South American hylid frogs. Gaps (−) have been introduced to maximize sequence similarities. Identical amino acids have *shaded backgrounds*. Carboxamidated amino acids are in *italics*. New names are proposed according to the frog species names set out in Amphibian species of the world (http://research.amnh.org/herpetology/amphibia/index.php) and [1]. *Phyllomedusinae* species: A, *annae*; B, *bicolor*; C, *callidryas*; DA, *dacnicolor*; DI, *distincta*; H, *hypochondrialis*; L, *lemur*; LI, *litodryas*; O, *oreades*; S, *sauvagii*; TA, *tarsius*; TR, *trinitatis*. Assignment numbers for paralogous peptides are fonction of their publications dates. DRS, DS = dermaseptin; DRP = dermaseptin-related peptide; DD = dermadistinctin; DRG = dermaseptin-gene related; IRP, isulin-releasing-peptide [6,11,12,20–22,29,32,52,67,81–91].

comprises a N-terminal preprosequence of ~50 residues that is remarkably conserved both within and between species (Table 3), while the C-terminal sequence corresponding to the mature peptides varies markedly [5–7]. The conserved region comprises a 22-residue signal peptide followed by an acidic intervening sequence that ends in a typical prohormone processing signal Lys-Arg that precedes the single downstream copy of the mature peptide. This conservation also extends into the 5′- and 3′-untranslated regions of the corresponding mRNAs. Preprodermaseptin mRNAs thus show a unique pattern of very high sequence conservation surrounding a region of very high sequence variability. The contrast between the strikingly conserved preproregion and the hyperdivergent mature peptides is one of the most extreme examples observed to date for homologous gene products within a single order of organisms. Moreover, the pattern of conserved and variable regions in skin antimicrobial peptide precursors is the opposite of that of conventional secreted peptides, suggesting that the conserved preproregion is important for the biology of the expressing cell.

Genes encoding Dermaseptin B2 and phylloxin appear to be highly-conserved in terms of structural organization with a small intron of 137- and 175-bp, respectively, located between exon 1 (encoding signal peptide and first three residues of the acidic intervening sequence) and exon 2 (remainder of acidic intervening sequence and mature peptide coding sequence through into 3′-non-translated region) [11–13]. The structural organization and intron/exon boundary are preserved in the gene that encodes gaegurin 4 (new nomenclature: brevinin-2EMd) from *Rana rugosa* (*Glandirana emeljanovi*) but with an intronic sequence of 3.5-kb [14]. Thus, the gene duplication events most likely involved complete genetic loci including intron and exons.

3.2. Molecular strategies to generate peptide diversity in frog skin

The remarkable similarity of preproregions of precursors that give rise to very different peptides in distantly related frogs indicates that the corresponding genes form a multigene family originating from a common ancestor. The vast number of different peptides encoded by this gene family reflects an unprecedented degree of gene diversification similar to that of the gene families that mediate interactions between organisms, such as immunoglobulins or venom-derived toxins [15]. Phylogenetic reconstructions indicated that the genes encoding peptides of the dermaseptin superfamily in Hylidae and Ranidae arose from a common ancestral locus, which subsequently diversified by several rounds of duplication and subsequent divergence of loci [6,7,16,17]. Most of the duplication events predated the radiations of ranins and of South American hylids and occurred before cladogenesis in a species ancestral to all these species and arose before the isolation of India and South America from Africa in a pan-Gondwanan common ancestor of these species 200–150 mya. Therefore, the diversity of modern antimicrobial peptide, neuropeptide, and hormone loci present in hylids and ranids reflects the divergence of these genes since the Mesozoic. Gene duplication was followed by focal hypermutation of the mature-peptide sequences. The abnormally high bias for transversional versus transitional mutations in the mature-peptide region of transcripts is the diagnostic fingerprint that provided evidence for targeted hypermutation of the mature peptide exon. The transversion/transition (Tv/Ts) ratio of 2 observed for the mature-peptide region of dermaseptin transcripts is similar to the in vitro transversion bias measured for the SOS-inducible polymerase V, and the low processivity of this polymerase [6,7]. These results may suggest that a targeted mutagenic process involving a DNA Pol V-like

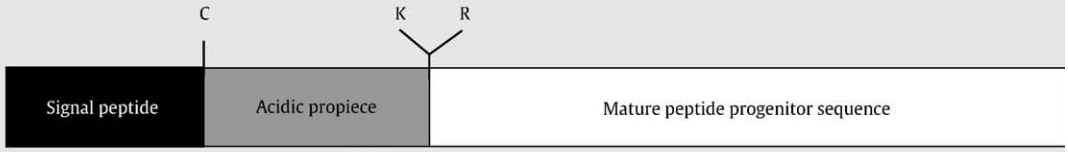
Table 2
The phylloseptin, plasticin, dermatoxin, phylloxin and hyposin families, and orphan peptides

Original name	Sequence	New name	Abbreviation
Phylloseptin			
PBN1 [6, 51]	FLSLIPHIVSGVAALAKH*L*	Phylloseptin-B1	PLS-B1
PHYLLOSEPTIN-1 [86]	FLSLIPHAINAVSAIAKH*N*	Phylloseptin-H1	PLS-H1
PHYLLOSEPTIN-2 [86]	FLS-IPHAINAVSTLVHH*F*	Phylloseptin-H2	PLS-H2
PHYLLOSEPTIN-3 [92]	FLSLIPHAINAVSALAN*H*	Phylloseptin-H3	PLS-H3
PHYLLOSEPTIN-6 [92]	---SLIPHAINAVSAIAKH*F*	Phylloseptin-H4	PLS-H4
PHYLLOSEPTIN-7 [92]	FLSLIPHAINAVSAIAKH*F*	Phylloseptin-H5	PLS-H5
PHYLLOSEPTIN-8 [92]	FLSLIPTAINAVSALAKH*F*	Phylloseptin-H6	PLS-H6
PHYLLOSEPTIN-9 [92]	FLSLLPSLVSGAVSLVKI*L*	Phylloseptin-H7	PLS-H7
PHYLLOSEPTIN-10 [92]	FLSLLPSLVSGAVSLVKK*L*	Phylloseptin-H8	PLS-H8
PHYLLOSEPTIN-11 [92]	FLGLLPSIVSGAVSLVKK*L*	Phylloseptin-H9	PLS-H9
PHYLLOSEPTIN-12 [52]	FLSLLPSIVSGAVSLAKK*L*	Phylloseptin-H10	PLS-H10
PHYLLOSEPTIN-13 [52]	FLSLIPHAINAVGVHAKH*F*	Phylloseptin-H11	PLS-H11
PHYLLOSEPTIN-14 [52]	FLSLIPAAISAVSALADH*F*	Phylloseptin-H12	PLS-H12
PHYLLOSEPTIN-15 [52]	LLSLVPHAINAVSAIAKH*F*	Phylloseptin-H13	PLS-H13
PHYLLOSEPTIN-L1 [90]	LLGMIPLAISAISALSK-*L*	Phylloseptin-L1	PLS-L1
PHYLLOSEPTIN-O4 [92]	FLSLIPHAINAVSTLVHH*S*	Phylloseptin-O1	PLS-O1
PHYLLOSEPTIN-O5 [92]	FLSLIPHAINAVSAIAKH*S*	Phylloseptin-O2	PLS-O2
Plasticin (PTC)		Plasticin-B1	PTC-B1
PBN2 [6, 51]	GLVTSLIKGAGKLLGGLFGSVTGGQ*S*	Plasticin-A1	PTC-A1
DRP-AA-2-5 [84]	GLVSGLLNTAGGLLGDLLGSLGSL*G*	lasticin-C1	PTC-C1
DRP-AC-1 [6]	GLLSGILNTAGGLLGNLIGSL*SN*---	in-C2	PTC-C2
DRP-AC-2 [6]	GLLSGILNSAGGLLGNLIGSL*SN*---	Plasticin-DA1	PTC-DA1
DRP-PD-3-6 [84]	GVVTDLLNTAGGLLGNLVGSL*G*---	Plasticin-S1	PTC-S1
DRS S10 [67]	GLVSDLLSTVTGLLGNLGGGGLKKI-		
		Dermatoxin B1	DRT-B1
Dermatoxin (DRT)		Dermatoxin A1	DRT-A1
DERMATOXIN [93]	SLGSFLKGVGTTLASVGKVVSDQFGKLLQAGQG	Dermatoxin DA1	DRT-DA1
DRP-AA-1-1 [84]	SLGSFMKGVGKGLATVGKIVADQFGKLLEAGQG	Dermatoxin S1	DRT-S1
DRP-PD-1-5 [84]	SLGSFMKGVGKGLATVGKIVADQFGKLLEAGKG		
DERMATOXIN S [94]	ALGTLLKGVGSAVATVGKMVADQFGKLLQAGQG	Phylloxin-B1	PLX-B1
		Phylloxin-S1	PLX-S1
Phylloxin (PLX)			
PHYLLOXIN [95]	GWMSKIASGIGTFLSGMQQG	Hyposin-H1	HPS-H1
PHYLLOXIN S [94]	GWMSKIASGIGTFLSGVQQG	Hyposin-H2	HPS-H2
		Hyposin-H4	HPS-H4
Hyposin (HPS)		Hyposin-H5	HPS-H5
HYPOSIN-1 [96]	LRPAVIRPK---G*K*--	Hyposin-H3	HPS-H3
HYPOSIN-2 [96]	LRPAFIRPK---G*K*--		
HYPOSIN-3 [96]	LRPAVIVRT--KG*K*--		
HYPOSIN-4 [96]	FRPALIVRT---KGTR*L*		
HYPOSIN-5 [96]	LGPALITRKPLKGK*P*-		
Orphan peptides			
DRP-PD-3-7* [84]	LLGDLLGQTSKLVNDLTDTVGSIV		
DRP-AA-3-4* [84]	GMWGSLLKGVATVVKHVLPHALSSQQS		
DRS S9* [67]	GLRSKIWLWVLLMIWQESNKFKKM		
	ENREVPPGFTALIKTLRKCKII (chain 1)		
Distinctin Di1 [97]	NLVSGLIEARKYLEQLHRKLKNCKV (chain 2)		

Phyllomedusinae species: A, *annae*; B, *bicolor*; C, *callidryas*; DA, *dacnicolor*; H, *hypochondrialis*; L, *lemur*; O, *oreades*; S, *sauvagii*. Assignment numbers for paralogous peptides are fonction of their publications dates. PLS, = phylloseptin; DRP = dermaseptin-related peptide; DRT, dermatoxin; PBN, *Phyllomedusa bicolor* new; PLX = phylloxin; HPS, hyposin. Cysteins participating to a disulfide bridge are in italics.
*Nomenclature for the orphan peptides awaits discovery of novel members [6,51,52,67,84,86,90,92–97].

Table 3
Canonical precursor arcitecture of peptides from the dermaseptin superfamily

A/



B/

Dermaseptin B1	MDILKKSLFLVLFLGLVSLSICEEEKRENEDEEK-Q-DDEQSEMKRAMWKDVLKKIGTVALHAGKAALGAVADTISQgEQ
Dermaseptin B2	MAFLKKSLFLVLFLGLVSLSICEEEKRENEDEEE-QEDDEQSEMKRGLWSKIKEVGKEAAKAAAKAAGKAALGAVSEAVgEQ
Dermaseptin B3	MAFLKKSVFLVLFLGLVSLSICEEEKREEENEEK-QEDDEQSEEKRALWKNMLKGIGKLAGQAALGAVKTLVGAE
Dermaseptin B4	MAFLKKSLFLVLFLGLVSLSICEEEKRENKDEIE-QEDDEQSEEKRALWKDILKNVGKAAGKAVLNTVTDMVNQgEQ
Dermaseptin B6	MAFLKKSLFLVLFLGLVSLSVCEEEKRENEDEME-QEDDEQSEEKRALWKDILKNAGKAALNEINQLVNQgEL
Dermaseptin B9	MAFLKKSLFLVLFLGLVSLSVCEEEKRENEDEEE-QEDDEQSEEKRALWKTIIKGAGKMIGSLAKNLLGQAPES
Dermaseptin SI	MDILKKSLFLVLFLGLVSLSICEEEKRENEDEEK-QEDDEQSEMKRALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ
Dermaseptin S6	MDILKKSLFFILFLGLVSLSISEEEKRENEDEED-QEDDEQSEEKRGLWSKIKTAGKEAAKAAAKAAGKAALNAVSEAIgEQ
Dermaseptin S7	MDILKKSLFLVLFLGLISLSFCEEEKRENEDEEE-QEDDEQSEEKRGLWKSLLKNVGKAAGKAALNAVTDMVNQgEQ
Dermaseptin S8	MDILKKSLFLVLFLGLVSLSICEEEKRENEDEEK-QEDDEQSEMKRALWKTMLKKLGTVALHAGKAALGAAADTISQgAQ
Dermaseptin S11	MAFLKKSLFLVLFLGMVSLSICEEEKRENEDEEE-QEDDEQSEEKRALWKTLLKGAGKVFGHVAKQFLGSQGQPES
Dermaseptin S12	MASLKKSLFLVLFLGLVSLSICEEEKRENEDEEN-QEDDEQSEMRRGLWSKIKEAAKTAGKMAMGWVNDMVgEQ
Dermaseptin S13	MAFLKKSLFLVLFLGLVSLSICDEEKRENEDEEN-QEDDEQSEMRRGLRSKIKEAAKTAGKMALGWVNDMAgE
Dermaseptin A3	MAFLKKSLFLVLLLGLISLSICEEEKRENEVEEE-QEDDEQSELRRSLWSKIKEMAATAGKAALNAVTGMVNQgEQ
Dermaseptin A4	MAFLKKSLFLVLFLGMVSLSICEEEKREEENE----QEDDEQSEEKRGMFTNMLKGIGKLAGQAALGAVKTLAgEQ
Dermaseptin A5	MAFLKKSLFLVLFLGLVSLSICEEEKRENEDEEE-QEDDEQSEMKRGMWSTIRNVGKSAAKAANLPAKAALGAISEAVgEQ
Dermaseptin C3	MAFLKKSLLLVLFLGLVSLSICEEEKRENEDEEE-QEDDEQSEMRRSVLSTITDMAKAAGRAALNAITGLVNQgEQ
Dermaseptin DA2	MALVKKSLFLVLFLGLVSLSICE-EKRENEDEEE-QEDDEQSEEKRALWKTLLKKVGKVAGKAVLNAVTNMANQNEQ
Dermaseptin DA3	MAFLKKSLFLVLFLGLVSLSICE-EKRENEDEEE-QEDDEQSEEKRGMWSKIKNAGKAAAKASKKAAGKAALGAVSEALgEQ
Phylloseptin B1	MAFLKKSLFLVLFLGLVSLSICEEEKRETEEKEYDQGEDDKSEEKRFLSLIPHIVSGVAALAKHLG
Plasticin S1	MAFLKKSLFLVLFLALVPLSICEEEKREGENEKE-QEDDNQSEEKRGLVSDLLSTVTGLLGNLGGGGLKKI
Plasticin B1	MAFLKKSLFLVLFLALVPLSICEE-KKSEEENEEKQEDD-QSEEKRGLVTSLIKGAGKLLGGLFGSVTGGQS
Plasticin A1	MAFLKKSLFLVLFLAIVPLSICEEEKREEENEEK-QEDDDQS--KRGLVSGLLNTAGGLLGDLLGSLGSLSGgES
Plasticin DA1	MAFLKKSLFLVLFLALVPLSICEAEKREEENEEK-QEDDDESEKKRGVVTDLLNTAGGLLGNLVGSLSGgER
Plasticin C1	MAFLKKSLLLVLFLGLVSLSICEEEKRENEDEEK-QEDDDQSENKRGLLSGILNTAGGLLGNLIGSLSNgES
Plasticin C2	MAFLKKSLLLVLFLALVPLSICEEEKREEEDEEK-QEDDDQSENKRGLLSGILNSAGGLLGNLIGSLSNgES
Dermatoxin S1	MAFLKKSLFLILFLGLVPLSFCENDKREGENEEE-QDDD-QSEEKRALGTLLKGVGSAVATVGKMVADQFGKLLQAGQG
Dermatoxin B1	MAFLKKSLFLVLFLGLVPLSLCESEKREGENEEE-QEDD-QSEEKRSLGSFLKGVGTTLASVGKVVSDQFGKLLQAGQG
Dermatoxin DA1	MAFLKKSLFLVLFLGLVPLFLCENEKREGENEKE--ENDDQSEEKRSLGSFMKGVGKGLATVGKIVADQFGKLLEAGKG
Dermatoxin A1	MAFLKKSLFLVLFLGLVPLFLCENEKREGENEKE--ENDDQSEEKRSLGSFMKGVGKGLATVGKIVADQFGKLLEAGQG
Phylloxin S1	MVFLKKSLLLVLFVGLVSLSICEENKREEHEEVE--ENAEKAEEKRGWMSKIASGIGTFLSGVQQg
Phylloxin B1	MVFLKKSLLLVLFVGLVSLSICEENKREEHEEIE--ENKE-AEEKRGWMSKIASGIGTFLSGMQQg
DRP-AA-3-4	MAFLKKSLFLVLFLGLVSLSICDEEKRENEDEEE-QEDDEQSEEKRGMWGSLLKGVATVVKHVLPHALSSQQS
DRP-PD-3-7	MSFMKKSLFLVLFLGVVSLSNCEEEKGE-ENEEDH-EEHH--EEKRLLGDLLGQTSKLVNDLTDTVGSIV
DRS S9	MAFLKKSLFLVLFLGLVSLSICDEEKRENEDEEN-QEDDEQSEMRRGLRSKIWLWVLLMIWQESNKFKKM

A/ Diagram of preprodermaseptin cDNAs. The coding region, including the signal peptide, the acidic propiece and the antimicrobial progenitor sequence is drawn as a rectangle. The signal peptide includes the first 22 amino acid residues, while the acidic propiece comprises 21–24 residues. B/ Examples of amino acid sequences of preprodermaseptins encoding antimicrobial peptides from South American hylid frogs. Identical amino acids have *shaded backgrounds*. Amino acids in *italics* are removed during processing of proforms to expose the extra Gly residue (*lower case*), which serves as an amide donor for the C-terminal residue of mature peptides. Peptides are named according to the new proposed nomenclature [1].

enzyme has operated in hylids and ranids within the peptide progenitor sequence of antimicrobial peptide loci, but not in the signal peptide and acidic propiece domains. Skin peptides do not participate in the general metabolism and physiology of the frog producing them. Since no deleterious effects are expected when a new peptide variant rapidly emerges by multiple changes in a current sequence, the new sequence may bypass the actions of neutral and negative (purifying) selections. There is strong evidence for diversifying selection (adaptative evolution, positive Darwinian selection) following dermaseptin gene duplication and focal hypermutation [6,7,16,18]. Comparisons of the proportions of nonsynonymous substitutions per nonsynonymous site (Dn) and proportions of synonymous substitutions per synonymous site (Ds) measured within the mature-peptide domain among all intraclade comparisons showed that Dn/Ds is commonly greater than 1 when Ds is less than 0.5, but less than 1 when Ds is greater than 0.5. Despite the extreme divergence of the sequences that impaired estimates of Dn and Ds due to the saturation of nucleotide substitutions, these results suggest that hylid and ranid loci have been subject to diversifying selection potentially within the mature-peptide domain. Thus, the combination of targeted hypermutation to generate great variation plus the subsequent action of positive selection may explain both the hypervariation and large number of peptides per species.

The evolutionary pressure which results in the conservation of the signal peptide and, albeit to a lesser extent, the acidic propiece in preprodermaseptins is especially striking in view of the extreme variations in the contiguous antimicrobial peptide domain and the very ancient history of the gene family. This suggests that these conserved elements have important functions. Conticello et al. have suggested that conserved elements in an otherwise hypermutable DNA sequence might be protected from mutagenesis by specifically bound macromolecules that serve to prime DNA Pol V-like polymerase in the vicinity [19]. By stopping normal DNA replication, DNA-bound macromolecules may then create single-strand gaps in the replicating strand, thus recruiting mutagenic polymerase as part of the damage response to the lesion. This scenario is attractive in that it provides a plausible explanation for the conservation of the signal peptide sequences for millions of years although it lies in the precursors of very different peptides produced by very distantly related species of frog.

Table 4
Antimicrobial activity of dermaseptins B against a selected panel of microorganisms[1]

Microorganisms	DRS B1	DRS B2	DRS B3
Mollicutes			
Spiroplasma apis	**0.3 (1.8)**	**0.3 (12.5)**	**3.1 (12.5)**
Spiroplasma citri	**0.4 (3.1)**	**0.4 (3.1)**	**3.1 (12.5)**
Spiroplasma floricola	**25 (100)**	**12.5 (R)**	**6.2 (50)**
Spiroplasma melliferum	**1.5 (6.25)**	**1.5 (6.25)**	**6.2 (25)**
Acholeplasma laidlawii	**3.1 (6.25)**	**3.1 (6.25)**	**3.1 (6.25)**
Mycoplasma gallicepticum	**R**[a]	**R**	**25 (100)**
Mycoplasma mycoides	**R**	**R**	**R**
Gracilicutes			
Escherichia coli K12	**1.5 (1.5)**	**1.5 (1.5)**	**1.5 (1.5)**
Pseudomonas aeruginosa	**6.2 (12.5)**	**12.5 (12.5)**	**3.1 (6.2)**
Salmonella typhimurium	**3.1 (3.1)**	**3.1 (3.1)**	**3.1 (3.1)**
Rhizobium meliloti	**0.3 (3.1)**	**0.3 (3.1)**	**0.8 (0.8)**
Pasteurella multocida	**25 (100)**	**12.5 (50)**	**25 (50)**
Firmicutes			
Staphylococcus aureus	**12.5 (25)**	**12.5 (25)**	**3.1 (3.1)**
Enterobacter faecalis	**50 (50)**	**50 (50)**	**12.5 (50)**
Bacillus megaterium	**0.3 (0.3)**	**0.3 (0.3)**	**0.8 (0.8)**
Corynebacterium glutamicus	**1.5 (1.5)**	**1.5 (1.5)**	**1.5 (1.5)**
Yeasts			
Saccharomyces cerevisiae	**5.5 (15)**	**5.5 (15)**	
Candida albicans	**10 (25)**	**5 (15)**	
Cryptococcus neoformans	**0.3 (15)**	**1.5 (1.5)**	
Fungi			
Microsporum canis	**0.8**	**1.5**	
Tricophyton mentagrophytes	**10 (25)**	**15 (30)**	
Arthroderma simii	**3.1 (3.1)**	**5 (15)**	
Aspergillus fumigatus	**3.1 (6.2)**	**3.1 (6.2)**	
Protozoa			
Leishmania mexicana	**3.1 (3.1)**	**3.1 (3.1)**	
Leishmania major	**5.5 (15)**	**1.5 (10)**	
Hemolysis[b]			
Human erythrocytes	>**250**	>**250**	>**100**

[1] The antimicrobial activity is expressed as MIC (μM), the minimal peptide concentration required for total inhibition of cell growth in liquid medium, and (LD), the peptide concentration required to kill the microorganisms.
[a] Strains were considered resistant (R) when their growth was not inhibited by peptide concentrations up to 100 μM.
[b] dose producing 100% hemolysis after 1 h incubation with the peptide.

3.3. A gene-based combinatorial peptide library

The outcome of massive gene duplication and focal hypermutation events is a collection of combinatorial peptide libraries, from which the subsequent positive selection may have retained either the most relevant peptides targeting specific microorganisms or a complex cocktail that enables frogs to survive in microbe-laden environments. Each ranin or hylid frog species produces its own set of antimicrobial peptides. Some of these peptides differ by only a few amino acid substitutions or deletions and have similar biochemical characteristics, i.e. dermaseptins B1 and B2. Other peptides have widely different sequences and physicochemical properties, i.e. dermaseptins and plasticins. The presence in frog skin of numerous antimicrobial peptides, acting separately or in concert, may have a selective survival value in habitats laden with microorganisms. Without direct functional characterization, it would have been difficult to predict the different spectra of these peptides. Moreover, these peptides act in synergy, with the mixture having up to a 10- to 100-fold greater antibiotic activity than the peptides separately [6,20]. Hence, the hypervariability of skin antimicrobial peptides and the proposed mechanisms of diversification could be part of a strategy for providing frogs with a maximum protection against a wide range of infectious microorganisms. Also, these antimicrobial peptides with such diverse structures and spectrum of action can be viewed as the successful evolution of a multi-drug defense system, which minimizes the chance of microorganisms developing resistance to individual peptides (see Section 7).

South American and Australian hylids, as well as Indian, European, Asian and North American ranins, have different patterns of distribution with respect to geography, climate, vegetation and habitats (aquatic, semi-aquatic, terrestrial, arboreal, torrential, fosserial, rocky), some of them showing very unusual and extreme adaptations. Targeted hypermutation of the C-terminal antimicrobial-coding region of preprodermaseptin genes might have evolved as a way of increasing genetic diversity and so accelerating the adaptation of frogs to noxious microbial fauna when microbial predators changed very rapidly with shifts to new ecological niches.

4. Dermaseptins (sensu stricto): canonical α-helical amphipathic peptides with broad-spectrum antimicrobial activity and additional biological functions

Peptides of the dermaseptin family are prototypical members of a large class of membrane-damaging cationic peptides that undergo coil-to-helix transition upon binding to lipid bilayers. The first member of the dermaseptin family, named dermaseptin S1, was isolated from an extract of dried pieces of skin of *Phyllomedusa sauvagei* in the early 1990s [21]. This 34-residue peptide has lytic activity against Gram-positive and Gram-negative bacteria, yeast, and protozoa, without harmful effects against mammalian cells. It was the first gene-encoded eukaryotic peptide to show lethal effect against filamentous fungi that are responsible for opportunistic infections that result from the immunodeficiency syndrome or the use of immunosuppressive agents. This was followed by the isolation of adenoregulin (also named dermaseptin B2) from *P. bicolor* skin, a peptide that interacts with the adenosine receptor [22], and dermaseptin B1 [23]. These 2 peptides were thought to be unrelated until attempts to clone their precursor polypeptides revealed the presence of a common preproregion and 5′- and 3′-UTRs [24]. Since then, additional members of the dermaseptin family were rapidly identified in various South American species.

Most of the peptides of the dermaseptin family have a very broad range of antimicrobial activity. The dermaseptins (sensu stricto) are cidal against a wide spectrum of microorganisms, including mollicutes, bacteria, fungi, protozoa, yeast and enveloped viruses. Despite high sequence similarities, the dermaseptins differ in their potency for killing the various agents (Table 4). It is noteworthy that the antimicrobial potencies are essentially independent of the bacterial envelope structure. The dermaseptins S exhibit synergy of action upon combination, resulting in some cases in a 100-fold increase in antibiotic activity of the mixture over the activity of the peptides separately [20]. Shortening the peptide chain of dermaseptin S3 to dermaseptin S3-[1–16]-NH_2 does not affect the potency of the peptide. Further reduction of the chain length yield derivatives gradually showing reduced activity. However, analogues as short as 10–12 residues in length remain fully active against several bacterial strains [25]. Dermaseptins S derivatives with improved toxicity profiles: a 28-residues [K^4, K^{20}]-dermaseptin S4 and 2 shorter versions [K^4]-dermaseptin S4-[1–16] and [K^4, K^{20}]-dermaseptin S4-[1–13] have been investigated for antibacterial activity in vivo, using a peritonitis model of mice infected with *Pseudomonas aeruginosa* [26]. Naïve mice exhibited 75% mortality, compared with 18% and 36% mortality in mice that received a single i.p. injection (4.5 mg/kg) of [K^4]-dermaseptin S4-[1–16] and [K^4, K^{20}]-dermaseptin S4-[1–13], respectively. Dermaseptins S1–S5 exhibit cidal activity against *Leishmania mexicana* in its promastigote form at μM doses [27] (Fig. 1). Immunocytochemical, freeze fracture, and label fracture microscopic observations show that the peptide generates major perturbations of the lipid bilayer, leading to death of the parasites. Dermaseptin S3 and

derivatives of dermaseptin S4 selectively disrupt the plasma membrane of the intracellular parasite *Plasmodium falciparum* without harming that of the mammalian host cell [28]. The resulting antimalarial activity is allegedly exerted after the harmless peptide binding to the membrane of the host cell, followed by peptide translocation across a number of intracellular membrane systems and interaction with that of the intraerythrocitic parasite. Dermaseptins DDL and DDK (DI2 and DI1 in the new proposed nomenclature) and O1 show antiprotozoan activity against *Trypanosoma cruzi* in its tryptomastigote and epimastigote forms cultivated in both cell culture and blood media, without toxicity against mouse erythrocytes and white blood cells [29]. Dermaseptins S1–S5 display antiviral activity against herpes simplex virus type I and HIV-1 virus at μM doses [30]. The most potent peptide, dermaseptin S4, inhibits cell-free and cell-associated HIV-1 infection of D4-CCR5 indicator cells and human primary T lymphocytes. The peptide is effective against R5 and X4 primary isolates and laboratory-adapted strains of HIV-1. Its activity is directed against HIV-1 particles by disrupting the virion integrity.

Most of the peptides belonging to the dermaseptin superfamily have been identified primarily by their antimicrobial activity. However, additional biological functions have been recognized that may, or may not be directly associated with pathogen clearance. For instance, adenoregulin (dermaseptin B2) was first isolated by Daly et al. as a peptide able to stimulate binding of agonists to A1 adenosine receptors [22], and was further shown to enhance the binding of agonists to several G-protein coupled receptors in rat brain membranes through a mechanism involving enhancement of guanyl nucleotide exchange at G-proteins, resulting in a conversion of receptors into a high affinity state complexed with guanyl nucleotide-free G-protein [31]. On the other hand, dermaseptin B4 significantly stimulates insulin release by 1.5 to 2.5-fold in acute incubations with glucose-responsive BRIN-BD 11 cells [32]. Dermaseptin S1 stimulates microbicidal activities of polymorphonuclear leukocytes [33]. Treatment of polymorphonuclear leukocytes with dermaseptin S1 (10–100 nM) stimulates production of reactive oxygen species and release of myeloperoxidase. In addition, low peptide concentrations (1–10 nM) prime the stimulation of respiratory burst induced by zymosan particles. The induced-burst is inhibited by selective protein kinase inhibitors, and is associated with early signaling events such as a rapid and transient elevation of cytosolic-free calcium concentration and phospholipase D activity. These data provide evidence of stimulating and priming properties of dermaseptin S1 on microbicidal activities of neutrophils, suggesting a potential role in modulating host-defense mechanisms.

The structure–activity relationships and mechanisms of antimicrobial action of dermaseptins have been extensively studied (see A. Mor, this issue, for an authoritative review). We will only review briefly some particular points. The mode of antimicrobial action of most dermaseptins is believed to be the permeation/disruption of the lipid plasma membrane of the target cells through a "carpet" mechanism [34,35]. Fluorescence-based studies using liposomes and surface plasmon resonance analysis of the interaction of dermaseptins with immobilized bilayers demonstrated that the peptides preferentially bind to negatively charged membranes with association affinity constants in the range of 10^7 M^{-1}. CD, FTIR spectroscopy, and 2-D NMR have shown that dermaseptins S, B, and O form an amphipathic α-helix in the presence of apolar solvents, or in the presence of SDS micelles or phospholipids vesicles (Fig. 2) [36–38]. Drs-B2 forms a well-defined amphipathic helix in SDS spanning residues 11–33 (Fig. 2). There is also a loose helical segment encompassing residues 3–8 that is separated from the well-defined helix by a small hinge region consisting of residues Val^9 and Gly^{10}. Helix 11–33 is totally amphipathic, that is, the apolar side chains (mostly Ala) are aligned on a portion of the helical cylinder, whereas the polar and charged residues (Lys) occupy the remaining surface. The position of Drs-B2 relative to the SDS micelle surface was investigated using paramagnetic probes. Titration with Mn^{2+} indicates that segments 8–12 and 24–33 are accessible to the paramagnetic ion. 5-doxyl spin-labeling experiments and fluorescence data show that residue Trp^3 in the N-terminal segment of the peptide lies inside the SDS micelles but remains close to the surface. Thus, these results suggest that Drs-B2 is mainly located at the interface between SDS micelles and water and that conformational flexibility of the N-terminal segment and the Val^9-Gly^{10} hinge region may facilitate the penetration of Trp^3 into the SDS micelles. The conformational versatility of the N-terminal segment led us to investigate its role in antimicrobial activity. The N-terminal truncated analogue Drs-B2-[10–33] is completely devoid of activity. Deleting the C-terminal peptide helix to give Drs-B2-[1–23] and Drs B2-[1–11] drastically decreased the antibacterial potency. This demonstrates that the N-terminal segment is necessary for antibacterial activity of

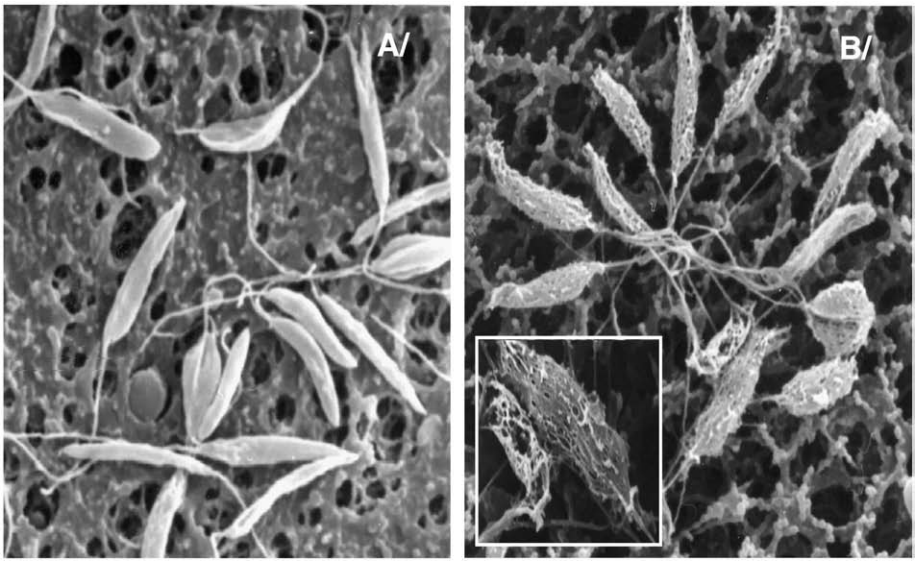


Fig. 1. Electron microscopic observations of *Leishmania mexicana* promastigotes (A) before and (B) after treatment by 5 μM dermaseptin S1. Within 5 min of incubation in the presence of Drs-S1, the flagellated parasites lost their motility. After treatment, the plasma membrane is peeled off (inset), and the microtubular network is the unique cell surface structure that still maintains the shape and integrity of the promastigote ghost. Drs-S1 inhibited promastigote growth by 50% at a concentration of 3 μM and by 100% at 10 μM.

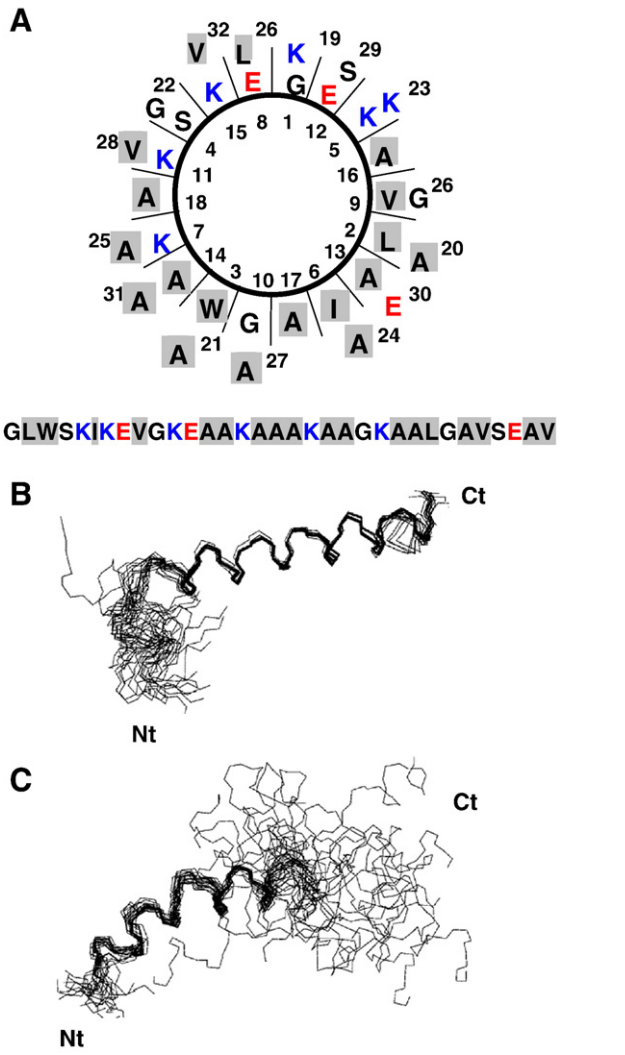


Fig. 2. (A) Dermaseptin B2 exhibits alternating hydrophobic and polar/charged residues along the primary structure and forms α-helical amphipathic helix in a membrane environment, with polar and apolar amino acids on opposing surfaces along the long axis of the helix. (B) NMR structures of dermaseptin B2 in 80 mM SDS. Family of 20 structures obtained from NMR data collected for 0.4 mM Drs-B2 in 80 mM SDS. Structures were superimposed by fitting the backbone atoms N, C, and C′ from residue 11 to residue 31. Drs-B2 forms a well-defined amphipathic helix in SDS spanning residues 11–33. There is also a loose helical segment encompassing residues 3–8 that is separated from the well-defined helix by a small hinge region consisting of residues Val[9] and Gly[10]. Helix 11–33 is totally amphipathic. (C) Family of 20 structures obtained from the NMR data determined in 20% TFE with 2 mM Drs-B2. Structures were superimposed by fitting the backbone atoms N, C, and C′ from residue 3 to residue 18.

Drs-B2. However, the entire peptide length is required for full permeabilization potency.

Comparison of the structures of Drs-B2 in 20% TFE and in 80 mM SDS also shows that the peptide conformations in these media differ significantly [37] (Fig. 2). The helical structure of Drs-B2 extends from Trp[3] to Ala[18] and is followed by a more flexible, loosely defined helix segment in TFE/water. This suggests that a negatively charged hydrocarbon–water interface is not essential for α-helix formation, but it is important for proper conformational adjustment to enable the peptide helix to interact optimally with the micelle surface. The electrostatic interactions between the positively charged lysine residues at the C-terminal domain of the peptide and the anionic SDS headgroups could drive primary binding and anchoring to the micelle surface.

According to the carpet-model, cationic peptides initially bind onto the negatively charged surface of the target membrane and cover it in a carpet-like manner. The high content of anionic lipids in prokaryotic membranes and their absence from the neutral matrix of erythrocytes account for the preferential binding of cationic peptides to the outer leaflet of bacterial bilayers through non-specific long-range electrostatic interactions. The four steps proposed to be involved in the model are: (i) binding of the cationic peptide to the phospholipid head groups, with subsequent formation of an amphipathic peptide helix; (ii) alignment of the helical peptides on the surface of the membrane so that their hydrophilic surface is facing the phospholipid head groups; (iii) rotation of the helical peptides leading to reorientation of the hydrophobic residues toward the hydrophobic core of the membrane. The resulting spatial segregation of polar and

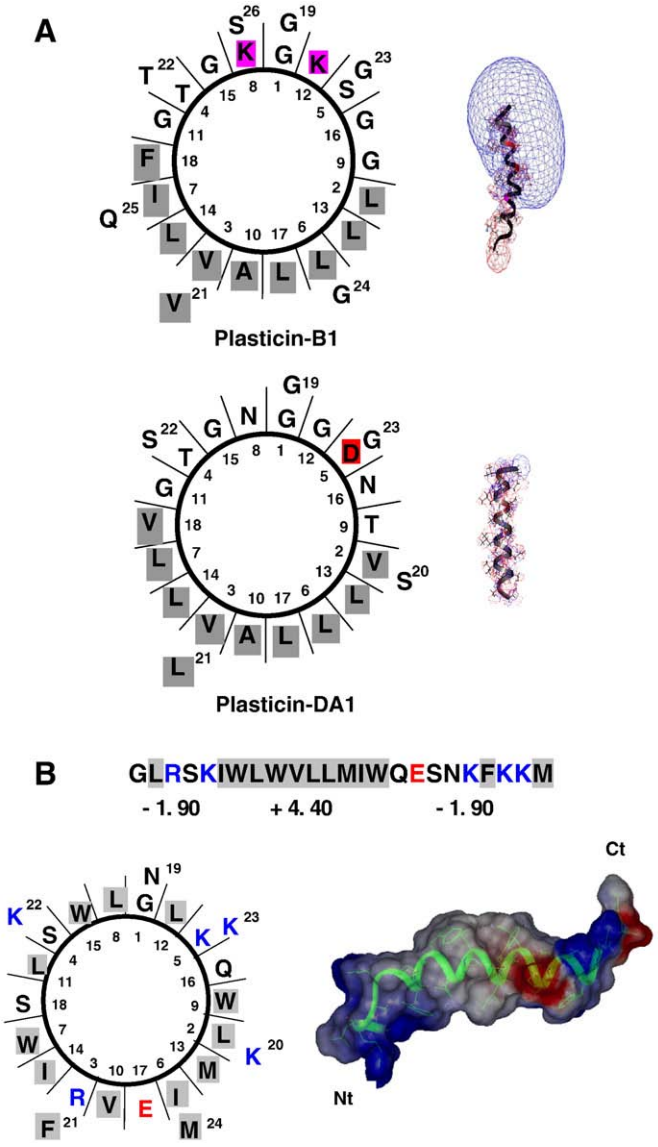


Fig. 3. (A) Helical wheel projections showing the distribution of hydrophilic and hydrophobic amino acids, and electrostatic diagrams of plasticins. Hydrophobic (gray background), positively charged (pink background), and negatively charged (red background) side chains are highlighted. The blue and red meshes represent the 1 kT/e positive and 1 kT/e negative electrostatic fields of the peptides calculated from the finite difference solution of the Poisson–Boltzmann equation. (B) Amino acid sequences, amphipathic properties and three-dimensional solution structure of dermaseptin S9 in 30% TFE. Dermaseptin S9 has a tripartite structure comprising a highly hydrophobic core sequence (mean hydrophobicity +4.40 determined by the Liu–Deber scale) flanked by hydrophilic and charged residues termini (mean hydrophobicity −1.90). Dermaseptin S9 forms α-helix in a membrane environment, but polar and apolar residues are not segregated on opposing surfaces along the long axis of the helix. The hydrophobic core is nonamphipathic because it has no hydrophilic residues.

apolar amino acids on opposing faces along the long axis of the helix permits insertion of a well-defined hydrophobic sector into the lipid bilayer. Note that the peptides do not become inserted deeply into the acyl core of the membrane because of the snorkeling of the regularly spaced lysine side chains that grip it to the membrane surface; and (iv) disintegration of the peptide-coated membrane by disrupting the bilayer curvature. As peptides cover the cell surface, transient holes may be formed. They may enable the passage of low molecular weight molecules prior to membrane lysis.

5. Plasticins: membrane-damaging peptides with "chameleon-like" structural and functional properties

The plasticins are a family of antimicrobial, orthologous peptides of the dermaseptin superfamily that have very similar amino acid sequences, hydrophobicities, and amphipathicities but differ markedly in their conformational plasticity and spectrum of activity [6]. This family includes the neutral plasticin-A1 (from *Agalychnis annae*), plasticin-C1 and plasticin-C2 (from *Agalychnis callidryas*), and plasticin-DA1 (from *Pachymedusa dacnicolor*), and the cationic plasticin-B1 (from *Phyllomedusa bicolor*) (Table 3). These 23–29-residue peptides are rich in glycine and leucine (29 and 25% on average) and contain 1 to 4 repeats of the glycine-zipper motif GXXXG (where X is any residue). Contrasting with amphipathic helical dermaseptins, plasticins display considerable conformational flexibility and polymorphism that modulates their ability to bind to and disrupt the bilayer membranes of prokaryotic and eukaryotic cells, and/or to reach intracellular targets.

5.1. The plasticins: orthologous peptides with different structural and functional properties

Plasticins have very similar amino acid sequences, but differ markedly in their net charge and activity spectra. For instance, plasticin-B1 with a net charge of +2, contains 2 positively charged lysine residues. These lysine residues are replaced by asparagines and glycine in plasticin-DA1. An additional aspartate residue in position 5 of plasticin-DA1 generates a peptide with a net charge of 0. The 2 peptides have identical overall hydrophobicities and amphipathicities, corresponding to a hydrophobic sector that subtends a radial angle of 180° on a helical wheel projection, in contrast to Drs-B2 presenting a radial angle of 120° (Fig. 3). Positive charge distribution differs for plasticins and Drs-B2. Drs-B2 appears uniformly positively charged, while the positives charges of plasticins are presented as N-terminal lobes. Glycine residues are predominant within the polar surface of the helix of plasticins, while leucine is predominant on the nonpolar surface. Despite their orthologous relationships and very similar sequences, the spectra of action of pasticin-B1 and plasticin-DA1 differ considerably (Table 5). Cationic plasticin-B1 was bactericidal against most of the tested microorganisms at μM concentrations and permeabilized the cytoplasmic membrane of *E. coli*, but neutral plasticin-DA1 was virtually inactive and did not permeate the bacterial membrane. However, both peptides have similar, albeit moderate hemolytic activities [39].

Incubation of HeLa cells with 100 μM plasticin-B1 induces cell necrosis after 3 h, whereas after 24 h, this plasticin at a concentration of 10 μM provokes damage in the plasma membrane and changes in cell morphology with local membrane fusion, formation of large polynucleated cells, and alteration of the nuclei, influencing condensation and fragmentation as attested by the presence of apoptotic bodies.

5.2. Bilayer lipid composition modulates the conformation of membrane-bound plasticins

Despite very similar amino acid sequences, plasticins adopted various conformations at anionic and zwitterionic membrane interfaces including helices, destabilized helix states, β-hairpin, β-sheet and disordered states (Table 5). Plasticin-B1 is mainly helical when bound to anionic DMPG or zwitterionic DMPC phospholipid vesicles,

Table 5
(A) Antimicrobial[a] and cytotoxic[b] activities of plasticins; (B) Membrane-bound conformation, membrane adsorption density, membrane perturbation of plasticins

(A) Microorganisms	DRP-PBN2	DRP-PD 3-6	(B)	DRP-PBN2	DRP-PD 3–6
Gram-negative bacteria				*DMPG*	
Escherichia coli B	6.25	R	*Membrane-bound conformation*		
Enterobacter cloacae	12.5	R	% helix	65	64
Klebsiella pneumoniae	6.25	R	% β-structure	0	9
Salmonella enteritidis	3.1	R	*DMPG adsorption density*		
Clostridium perfringens	50	R	$ng.nm^{-2}$ [e]	2.8	0.1
Listeria monocytogenes	25	R	PL ($ng.mm^{-2}$) [f]	1.2	0
Neisseria meningitidis	50	R	*Membrane perturbation*		
Vibrio Cholerae	25	R	Membrane interface (%) [g]	23	17
Pseudomonas aeruginosa	3.1	R	Hydrophobic core (%) [h]	5	4
Salmonella typhimurium	3.1	R			
Gram-positive bacteria					
Staphylococcus haemolyticus	50	300		*DMPC*	
Staphylococcus aureus	6.25	250	*Membrane-bound conformation*		
Streptococcus pneumoniae	6.25	nd [d]	% helix	47	16
Bacillus megaterium	25	R	% β-structure	27	62
Burkholderia cepacia	R [c]	R	*DMPC adsorption density*		
Yeasts			$ng.nm^{-2}$ [e]	1.2	0.2
Candida albicans	50	R	PL ($ng.mm^{-2}$) [f]	0.3	0.1
Saccharomyces cerevisiae	50	R	*Membrane perturbation*		
% hemolysis at 50 μM	30	42	Membrane interface (%) [g]	13	0
HeLa cells[h]	++	nd	Hydrophobic core (%) [h]	4	0

[a] The antimicrobial activity is expressed as MIC (μM), the minimal peptide concentration required for total inhibition of cell growth in liquid medium.
[b] Evaluated by video microscopy.
[c] Strains were considered resistant when their growth was not inhibited by peptide concentrations up to 400 μM.
[d] Not determined.
[e] Adsorption density obtained by surface plasmon resonance.
[f] PL represents the complex between peptide and membrane corresponding to the penetration of the peptide into the hydrophobic core of the bilayer as quantified after 10 min of desorption by SPR.
[g] 1727/1742 ratio increase (%) corresponding to membrane interface (νCO) determined by Fourier transform infrared spectroscopies.
[h] Percentage of νASCH modification compared to pure phospholipids established by FTIR spectroscopies.

but the contribution of β-sheet structures increases when mixed to zwitterionic vesicles. Plasticin-DA1 adopts a helical structure when bound to anionic vesicles but is β-sheeted in the presence of zwitterionic phospholipids. CD spectroscopy and NH/ND-exchange kinetics obtained by ATR-FTIR showed that plasticin-DA1 self-associates when bound to DMPG vesicles [39]. Molecular dynamics simulations performed in apolar medium starting from an ideal helix, demonstrated that plasticin-DA1 underwent transitions between three conformations (helix/coil/β-structure), while cationic plasticin-B1 oscillated between helix and disturbed helix. These observations shed light on the differences in structural malleability of closely related peptides and revealed the potential foldability within β-hairpin conformations of plasticins [39,40].

It has often been reported that antimicrobial potency is associated with an amphipathic structure, being either an α-helix or a β-sheet [41–43]. While all plasticins displayed a pronounced amphipathic character when modelled as a helix, this property was completely disrupted when peptides are represented in β-structures. Disruption of amphipathicity in β-sheeted conformations (either hairpin shaped or extended) may be a key for explaining differential potencies. Membrane-bound cationic plasticin-B1 exhibits antimicrobial activity when displaying an α-helix/β-structure ratio ≥ 1. In contrast, neutral plasticin-DA1 has an α-helix/β-structure ratio <1 when bound to phospholipid membranes, and is devoid of antimicrobial activity (Table 5).

5.3. The glycine-zipper motif GXXXG and self-association of plasticins

Selectivity and potency of antimicrobial peptides may additionally depend on properties other than their lipid-binding characteristics. Peptides need to cross the cell wall before reaching the cytoplasmic membrane, a process that depends on the structure and oligomeric state of the peptide. It has also been proposed that some peptides that disrupt membranes via a carpet mechanism approach the membrane in an oligomeric state and have a highly helical arrangement before coming in contact with the membrane [44]. The amino acid sequences of plasticins resemble those of transmembrane protein segments, and encompass several GXXXG motifs that are known to mediate interactions between transmembrane or fusion peptide helices [45]. The impact of GXXXG motifs on plasticin self-association was assessed by SDS-PAGE and measurement of translational diffusion coefficients by pulse field gradient NMR experiments (unpublished data). Although these experiments revealed the existence of a monomer–dimer equilibrium, the GXXXG motifs were not shown to promote strong oligomerization of plasticins to the same extent as the dimeric transmembrane segment of glycophorin A [45]. Other factors may be involved, such as the sequence context or intrinsic conformational flexibility.

5.4. Antimicrobial activity of cationic plasticins and interaction with anionic membranes

Cationic plasticin-B1 is mainly helical when bound to anionic DMPG mimetic membranes but can also adopt alternative conformations including β-hairpin-like structures at this interface [39,46] (Table 5). This is in contrast to magainin and cecropin, which also adopt helical structures at an air–water interface, whereas melittin only adopts a helical structure in the presence of lipids [47]. Plasticin-B1 forms peptide multilayers when adsorbed onto DMPG bilayer and binds to anionic membranes in a two-step process (Table 5). In a first step, the peptide binds to the membrane surface because of the hydrophobic effect and several non-specific interactions. In a second step, the subsequent formation of the peptide helix result in tightly bound peptide–lipid complexes that are primarily stabilized by the aliphatic and aromatic residues. Plasticin-B1 interacts with DMPG by decreasing the associated water content of the phospholipid heads, replacing water molecules by peptides themselves [39]. This suggests that the peptide may act via other ways than the breakdown of cytoplasmic membrane permeability. The interaction of the peptide with alkyl chains of DMPG phospholipids results in noticeable disordering of the acyl chain region of the fluid bilayer, probably due to gel to liquid–crystalline phase transition, or as a consequence of peptide insertion [48,49]. The abilities of plasticins to act on lipid membranes were also related to their potential to cross membranes, thus targeting the cytoplasm or nucleus. Consequently, plasticin-B1, giving the highest complex density in a DMPG bilayer (Table 5), is believed to have the strongest capacity to cross membranes.

5.5. Cytotoxic activity of cationic plasticins and interaction with zwitterionic membranes

Cationic plasticin B1 is mainly helical when bound to zwitterionic DMPC phospholipid vesicles, although the β-sheet structure contribution increases (Table 5). In contrast, it forms peptide multilayers when adsorbed onto the DMPC bilayer and binds to zwitterionic membranes in a two-step process as for anionic membranes. Nevertheless the peptide–lipid complex density is weaker, indicating a lower affinity for DMPC bilayers compared to DMPG. Plasticin-B1, mainly in α-helix conformation, is adsorbed to the same extent as dermaseptin B2 [50]. FTIR spectroscopy revealed that the binding of the peptides at the interface of the zwitterionic membrane results in membrane dehydration, formation of peptide–membrane hydrogen bonds and alteration of the $\nu AS(CH_2–CH_3)$ modes, indicating that the peptide interacts with the bilayer core (Table 5). Altogether, these biophysical data demonstrate the ability of cationic plasticin-B1 to oligomerize in zwitterionic membranes, partially through gain of β-structures. Beta structure contents could thus be used as an indicator of cytotoxic potency in the plasticin family.

5.6. Membrane-active neutral plasticins

A few membranotropic frog skin peptides bearing no net charge have been reported in the literature [51–54]. These peptides were found to be inactive against bacterial strains in vitro. The lack of measurable growth-inhibitory activity of neutral peptides toward bacteria is consistent with the proposal that the positive charge on the peptide is important for binding to the negatively charged phospholipids of the bacterial cell membrane. Nevertheless, it has been shown that neutral frog skin peptides can have a potent activity against microorganisms that the frog may encounter in the wild [55,56]. The fact that the structure of plasticin-DA1 differs greatly in the presence of anionic or zwitterionic vesicles demonstrates that this peptide has versatile conformations at membrane surfaces, i.e., the same amino acid sequence can have several conformations, depending almost exclusively on the lipid molecular environment. Thus, an amphipathic helical structure is not sufficient, in this case, for bacterial membrane disruption. Neutral plasticin-DA1, which interacts with anionic or zwitterionic phospholipid vesicles, mainly via hydrophobic interactions [39], is only weakly adsorbed in the micromolar range of antibacterial activity concentrations (Table 5). The lack of a positive net charge did not prevent peptide adsorption, but could prevent further rearrangements in the bilayer to form stable peptide–lipid complexes with lifetimes compatible with biological activity. Although plasticin-DA did not produce bacterial membrane leakage [6], it perturbes the peptide-DMPG vesicle interface and bilayer alkyl chains at millimolar concentrations, suggesting insertion into bacterial membranes. Surprisingly, neutral plasticin-DA1 induces no perturbation on DMPC vesicles, while it presents hemolytic activities in vitro. During bacterial attack, many peptides can be retrieved in frog skin secretions. The resulting cocktail contains simultaneously neutral, anionic, and cationic peptides. Since inactive neutral peptides enhance the activity of cationic peptides, they may act as primary membrane-disturbing peptides by adopting a transmembrane orientation.

Fig. 4 represents a schematic representation of structural inter-conversions and self-association in the presence of anionic membrane for plasticin-B1. Fluctuations in the antimicrobial potency appear to be related to shifts within the autoassociation stage altering the sequentiality of peptide oligomerization. Plasticins illustrate the various ways a peptide could perturb biomembranes, leading to a biological activity. As such, plasticins can be used as a general model to explore the impact of the structural malleability of membrane-active peptides (antimicrobial, cell-penetrating, and fusion peptides) on their ability to bind to and disrupt membranes of prokaryotic and eukaryotic cells, and/or to reach intracellular targets.

6. Dermaseptin S9: an amyloidogenic peptide with antimicrobial and chemoattractant properties

Dermaseptin S9 (Drs-S9), GLRSKIWLWVLLMIWQESNKFKKM, does not resemble any of the cationic and amphipathic antimicrobial peptides identified to date in having a tripartite structure that includes a highly hydrophobic core sequence encompassing residues 6–15 flanked at both termini by cationic and polar residues (Fig. 3). This structure is reminiscent of that of synthetic peptides originally designed as transmembrane mimetic models and that spontaneously become inserted into membranes [57,58].

6.1. Amyloid-like properties of Dermaseptin S9

Circular dichroism, FTIR and ATR-FTIR spectroscopies combined with surface plasmon resonance study show that Drs-S9 is structured as stable and ordered β-sheet aggregates in aqueous buffers, or once bound to anionic or zwitterionic phospholipid vesicles. Drs-S9 freshly dissolved in water or in PBS migrated with apparent molecular masses of 6 kDa and 12 kDa in SDS-PAGE gels suggesting that Drs-S9 could be self-associated as dimers or tetramers [59–61] (Fig 5). These structures slowly assembled into amyloid-like fibrils of 8 nm of diameter in aqueous environments *via* spherical intermediates that were visualized after 3 days and 7 days of incubation at 37 °C, respectively by electron microscopy and Congo red staining (Fig. 5). Furthermore, the self-associating properties of Drs-S9 were also corroborated by slow NH/ND-exchange kinetics monitored by ATR-FTIR [61].

Amyloidogenic peptides self-assemble, probably through specific molecular interaction patterns, generating ordered mature fibrils [62]. It is thought that due to the multiple aromatic residues present in amyloid-like peptides, π–π stacking plays a crucial role in the fibrillization process, by reducing the energetic barrier [63,64]. Since Drs-S9 bears a hydrophobic core-sequence (residues 8–15) rich in aromatic residues, the peptide can potentially establish π–π interactions [65] that could be at the origin of its amyloid-like behavior. The structural properties of most of the amyloidogenic peptides are environment-dependent. For example, $A\beta_{1-42}$ is α-helical in the presence of TFE [66], while it is poorly structured and aggregated in water, and β-sheet structured in phosphate buffer solutions. In the same way, Drs-S9 adopted a non-amphipathic α-helical conformation in TFE/water mixtures, but was highly aggregated in aqueous solutions and in the presence of SDS micelles (Fig. 3) [67].

6.2. Dermaseptin S9 induced chemotaxis via a seven -transmembrane G protein-coupled cell surface receptor

Drs-S9 triggered the chemotactic migration of human neutrophils, T lymphocytes and THP-1 monocytes with a maximal response for a concentration of 50 μM. Drs-S9 chemotactic activity seemed to be only partially sensitive to pertussis toxin and to ERK1/ERK2-dependent signaling. The cells' desensitization to Drs-S9 following a pre-treatment with $A\beta_{1-42}$ or fMLP suggests that Drs S9 could partially induce chemotaxis through the seven-transmembrane G_α protein-coupled FPRL1 receptor [68], and through an additional non-PTX sensitive receptor. Only few studies dealing with chemoattractive properties have been reported for frog antimicrobial peptides. They are all related to amphipathic α-helical peptides, such as Drs-S1 [33] or temporin A and analogues [69]. Drs-S1 and temporin A were shown to be chemotactic at 10 and 250 nM, respectively, contrasting with Drs-S9 that was found to induce the directional migration of neutrophils, monocytes and T lymphocytes within the same range of concentration reported for $A\beta_{1-42}$ [68]. Moreover, Drs-S9-induced chemotaxis results from a β-structured peptide, whereas the α-helical Drs-B2 had a poor chemotactic potential under similar conditions.

6.3. β-amyloid-like behavior of Dermaseptin S9 modulates its chemotactic and antimicrobial activities

Antimicrobial and chemotactic activities of Drs-S9 are modulated by its amyloid-like properties. Drs-S9 induced cell migration with a maximum efficiency for freshly dissolved peptide. Drs-S9 dissolved in

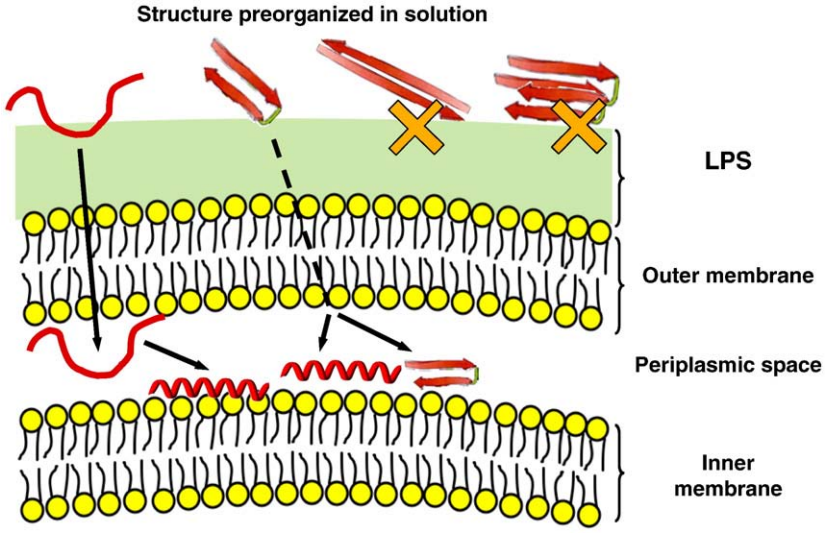


Fig. 4. Schematic view of the impact of preorganized structure of cationic plasticin-B1 and structural inter-conversions at anionic membrane interface for membrane-disturbing properties and antimicrobial potency.

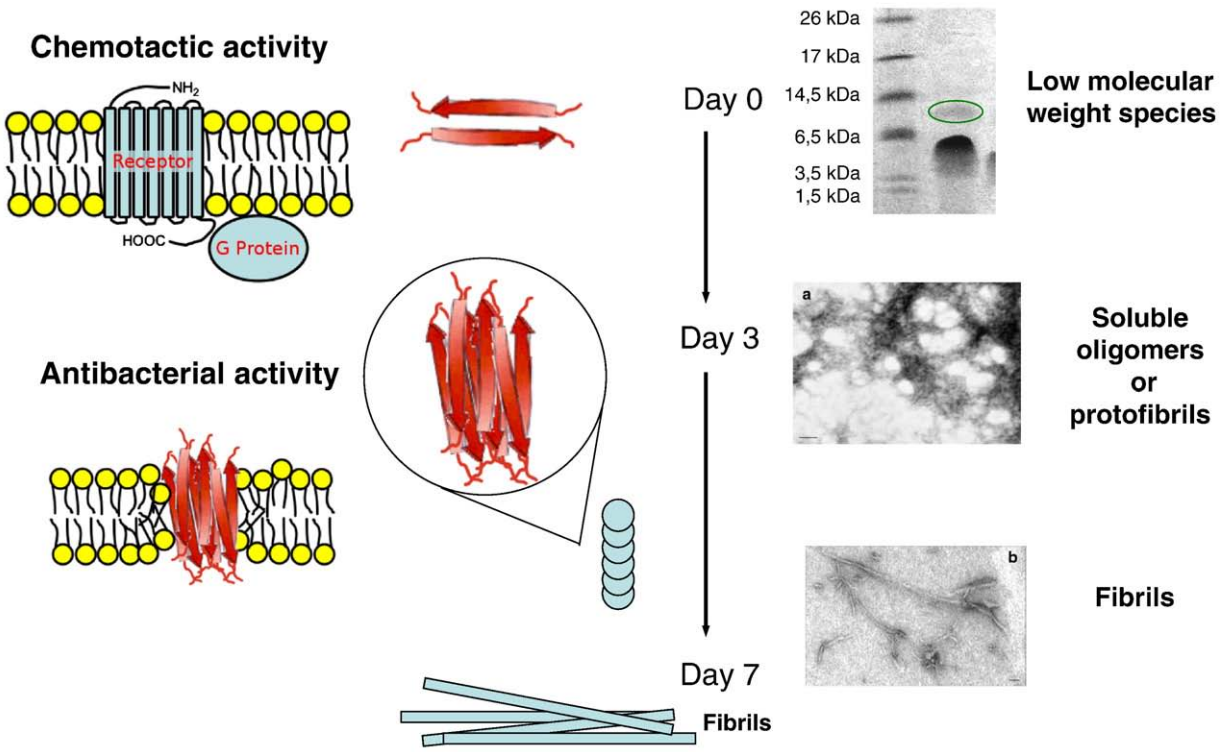


Fig. 5. Summary of the main oligomerization states adopted by Dermaseptin S9 in relation with its biological activities. At day 0, freshly dissolved Drs-S9 is mainly dimeric or tetrameric (soluble oligomers) and exhibits maximal chemotactic activity. At day 3, Drs-S9 is associated into cytotoxic spherical oligomers with a potent antimicrobial activity. At day 7, Drs-S9 forms in amyloid-like fibrils.

PBS, exhibited an antimicrobial activity against all Gram-negative bacteria strains tested at day 0. The antibacterial activity of 3 days-aged Drs-S9 in PBS (Table 6) was stronger than that of Drs-S9 at day 0. After 7-days of incubation in PBS at 37 °C, the peptide exhibited little or no antibacterial activity. Moreover, after removing the Drs-S9 from the wells and replacing it with fresh LB medium overnight, none of the sensitive strains was capable of resuming growth, suggesting that Drs-S9 is a bactericidal agent. Altogether, these data point out that Drs-S9 is a more potent bactericidal agent in its oligomeric spherical form detected at 3 days of incubation at 37 °C while it is a potent chemoattractant in its soluble, low-molecular weight self-associated forms in the same range of concentration as amyloidogenic peptides $A\beta_{1-42}$ and $PrP_{106-126}$.

6.4. Mechanisms of antimicrobial activity

The amyloidogenic properties of Drs-S9 influence its antibacterial activity suggesting different modes of membrane permeabilization. Drs-S9 has a high affinity for anionic model membranes that mimic bacterial membranes [67]. FTIR data underlined that anionic phospholipid vesicles promoted Drs-S9 structural interconversions from a β-hairpin into a β-sheet structure. As a consequence, the interaction of the peptide with alkyl chains of DMPG phospholipids resulted in a noticeable disturbance of the alkyl chain order of the fluid bilayer, probably as a consequence of Drs-S9 insertion. These data suggest that oligomeric β-sheet structured Drs-S9 exerted its microbicidal activity by perturbing both the membrane interface and the hydrophobic core of the bacterial membrane (Fig. 5).

It has been demonstrated that IAPP and α-synuclein protofibrils have the ability to permeabilize synthetic vesicles by a pore-like mechanism [70]. Interactions of amyloid $A_{\beta\ 25-35}$ peptide with phospholipids were also shown to be based on electrostatic interactions. These interactions are thought to favor the aggregation of the peptides, and the presence of the aggregates may disturb the lipid–water interface of the membrane [71]. While monomeric or weakly self-associated amyloidogenic peptide $A\beta_{1-40}$ binds rapidly but reversibly to liposomes, its aggregated form binds slowly but essentially irreversibly following a 2-step model: partial reversible adsorption of monomeric $A\beta_{\ 1-40}$, followed by an acceleration of peptide aggregation at the membrane interface, leading to some degree of irreversible adsorption. A similar behavior could be attributed *a posteriori* to Drs-S9 on DMPG vesicles, as assessed by SPR experiments using L1 sensorchip [67]. In contrast, Drs-S9 was irreversibly adsorbed on hydrophobic HPA support whatever its aggregation state. This kind of behavior is also opposed to that of peptides forming supramolecular protein–lipid amyloid-like fibers upon binding to negatively charged phospholipid-containing membranes [72–74]. On the other hand, Dermaseptins S were shown to be active against bacteria and to form aggregates at high peptide/lipid ratios, whereas Dermaseptins B are peculiarly active against fungi by forming aggregates at low peptide/lipid ratios [75]. This led to the proposal that the peptide aggregation state in solution could be an important factor affecting selective membrane disruption and cytoxicity. In conclusion, the dual abilities of Drs-S9, killing bacterial

Table 6
Effect of aging on the antimicrobial activity of dermaseptin S9 and dermaseptin B2

Bacterial strains	MIC (μM)			
	Dermaseptin S9			Dermaseptin B2
	Day 0	Day 3	Day 7	0, 3, and 7 days
Citrobacter rodentium	12.5	6.5	50	/
Salmonella typhimurium	25	6.5	100	3.1
Escherichia coli EPEC	50	12.5	100	3.1
Escherichia coli JPN15	50	25	R	0.2

Peptides were freshly dissolved in, or incubated during 3 or 7 days in PBS or H_20 at 37 °C before testing. The antimicrobial activity is expressed as MIC (μM), the minimal peptide concentration required for total inhibition of cell growth in liquid medium. Strains were considered resistant (R) when their growth was not inhibited by peptides concentrations >100 μM.

pathogens and activating immunological response, could be of great interest in the design of immunotherapeutic tools.

7. Concluding remarks

Organisms that produce venomous peptides for prey capture, defense, or competitor deterrence are very diverse, including spiders, scorpions, amphibians, cone snails and snakes. The pharmacological inventory encoded in the genomes of these animals is extraordinary [76]. It has been estimated that cone snails express 50,000 different neurotoxins based on a species count of 500 and a toxic repertoire of 100 peptides per venom [77,78]. Scorpions have been estimated to encode a similar diversity of peptide neurotoxins (100,000) based on a pharmacological repertoire of 70 peptides per venom and a species count of 1500 [79]. Spiders, with 80,000 estimated species and 50 peptides per venom lead to a total of 4 million spider-venom polypeptides. As stated by Olivera [80], gene products that mediate interactions between organisms are encoded by exogenes, a term used to rationalize conotoxin gene hypermutation. Exogene families that are targeted to other organism in the environment, such as the conopeptide genes, have to diversify very rapidly because each species has its own ecological niche. Thus, the evolutionary history of these genes would be expected to be strikingly different from the rest of the genome.

This suggest that spiders, scorpions, cone snails and hylid frogs, have independently evolved a similar mechanism to diversify their repertoire of toxic polypeptides by duplication of ancestral gene(s) followed by action of a hypervariability-generating mechanism that increases the rate of mutation of the mature-toxin loci. The pool of resulting paralogous genes was then subject to adaptive evolution to select useful variants, enabling these animals to perform a comprehensive search of pharmacophore space. [76]. Accordingly, inactive paralogs, such as plasticin-AD1, may be part of a bank of variants assembled by random fixation of non-synonymous mutations, which are available for further diversifying selection when the microbial environment or the genetic background changes.

References

[1] M. Amiche, A. Ladram, P. Nicolas, A consistent nomenclature of antimicrobial peptides isolated from frogs of the subfamily Phyllomedusinae, Peptides, In press, Accepted Manuscript, Available online 3 July 2008. (doi:10.1016/j.peptides.2008.06.017) <http://dx.doi.org.gate1.inist.fr/10.1016/j.peptides.2008.06.017>.

[2] P. Nicolas, M. Amiche, The dermaseptins, in: A.J. Kastin (Ed.), Handbook of Biologically Active Peptides, Elsevier, 2006, pp. 295–304.

[3] M. Amiche, F. Ducancel, A. Mor, J.C. Boulain, A. Menez, P. Nicolas, Precursors of vertebrate peptide antibiotics dermaseptin B and adenoregulin have extensive sequence identities with precursors of opioid peptides dermorphin, dermenkephalin, and deltorphins, J. Biol. Chem. 269 (1994) 17847–17852.

[4] M. Simmaco, G. Mignogna, D. Barra, F. Bossa, Antimicrobial peptides from skin secretions of *Rana esculenta*. Molecular cloning of cDNAs encoding esculentin and brevinins and isolation of new active peptides, J. Biol. Chem. 269 (1994) 11956–11961.

[5] M. Amiche, A.A. Seon, T.N. Pierre, P. Nicolas, The dermaseptin precursors: a protein family with a common preproregion and a variable C-terminal antimicrobial domain, FEBS Lett. 456 (1999) 352–356.

[6] D. Vanhoye, F. Bruston, P. Nicolas, M. Amiche, Antimicrobial peptides from hylid and ranin frogs originated from a 150-million-year-old ancestral precursor with a conserved signal peptide but a hypermutable antimicrobial domain, Eur. J. Biochem. 270 (2003) 2068–2081.

[7] P. Nicolas, D. Vanhoye, M. Amiche, Molecular strategies in biological evolution of antimicrobial peptides, Peptides 24 (2003) 1669–1680.

[8] T. Chen, C. Shaw, Cloning of the (Thr^6)-phyllokinin precursor from *Phyllomedusa sauvagei* skin confirms a non-consensus tyrosine O-sulfation motif, Peptides 24 (2003) 1123–1130.

[9] T. Chen, D.F. Orr, M. O'Rourke, C. McLynn, A.J. Bjourson, S. McClean, D. Hirst, P. Rao, C. Shaw, *Pachymedusa dacnicolor* tryptophyllin-1: structural characterization, pharmacological activity and cloning of precursor cDNA, Regul. Pept. 117 (2004) 25–32.

[10] P. Nicolas, M. Camier, M. Lauber, M.J. Masse, J. Möhring, P. Cohen, Immunological identification of high molecular weight forms common to bovine neurophysin and vasopressin, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 2587–2591.

[11] V. Vouille, M. Amiche, P. Nicolas, Structure of genes for dermaseptins B, antimicrobial peptides from frog skin. Exon 1-encoded prepropeptide is conserved in genes for peptides of highly different structures and activities, FEBS Lett. 414 (1997) 27–32.

[12] Y. Fleury, V. Vouille, L. Beven, M. Amiche, H. Wróblewski, A. Delfour, P. Nicolas, Synthesis, antimicrobial activity and gene structure of a novel member of the dermaseptin B family, Biochim. Biophys. Acta 1396 (1998) 228–236.

[13] T. Chen, R. Gagliardo, B. Walker, M. Zhou, C. Shaw, Partial structure of the phylloxin gene from the giant monkey frog, *Phyllomedusa bicolor*: parallel cloning of precursor cDNA and genomic DNA from lyophilized skin secretion, Peptides 26 (2005) 2624–2628.

[14] S.Y. Kwon, B.A. Carlson, J.M. Park, B.J. Lee, Structural organization and expression of the gaegurin 4 gene of *Rana rugosa*, Biochim. Biophys. Acta, 1492 (2000) 185–190.

[15] B.M. Olivera, R.W. Teichert, Diversity of the neurotoxic Conus peptides: a model for concerted pharmacological discovery, Mol. Interv. 7 (2007) 251–260.

[16] T.F. Duda Jr, D. Vanhoye, P. Nicolas, Roles of diversifying selection and coordinated evolution in the evolution of amphibian antimicrobial peptides, Mol. Biol. Evol. 19 (2002) 858–864.

[17] J.A. Tennessen, M.S. Blouin, Selection for antimicrobial peptide diversity in frogs leads to gene duplication and low allelic variation, J. Mol. Evol. 65 (2007) 605–615.

[18] J.A. Tennessen, Enhanced synonymous site divergence in positively selected vertebrate antimicrobial peptide genes, J. Mol. Evol. 61 (2005) 445–455.

[19] S.G. Conticello, Y. Gilad, N. Avidan, E. Ben-Asher, Z. Levy, M. Fainzilber, Mechanisms for evolving hypervariability: the case of conopeptides, Mol. Biol. Evol. 18 (2001) 120–131.

[20] A. Mor, P. Nicolas, Isolation and structure of novel defensive peptides from frog skin, Eur. J. Biochem. 219 (1994) 145–154.

[21] A. Mor, V.H. Nguyen, A. Delfour, D. Migliore-Samour, P. Nicolas, Isolation, amino acid sequence, and synthesis of dermaseptin, a novel antimicrobial peptide of amphibian skin, Biochemistry 30 (1991) 8824–8830.

[22] J.W. Daly, J. Caceres, R.W. Moni, F. Gusovsky, M. Moos Jr, K.B. Seamon, K. Milton, C. W. Myers, Frog secretions and hunting magic in the upper Amazon: identification of a peptide that interacts with an adenosine receptor, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 10960–10963.

[23] A. Mor, K. Hani, P. Nicolas, The vertebrate peptide antibiotics dermaseptins have overlapping structural features but target specific microorganisms, J. Biol. Chem. 269 (1994) 31635–31641.

[24] M. Amiche, F. Ducancel, E. Lajeunesse, J.C. Boulain, A. Ménez, P. Nicolas, Molecular cloning of a cDNA encoding the precursor of adenoregulin from frog skin. Relationships with the vertebrate defensive peptides, dermaseptins, Biochem. Biophys. Res. Commun. 191 (1993) 983–990.

[25] A. Mor, P. Nicolas, The NH2-terminal alpha-helical domain 1–18 of dermaseptin is responsible for antimicrobial activity, J. Biol. Chem. 269 (1994) 1934–1939.

[26] S. Navon-Venezia, R. Feder, L. Gaidukov, Y. Carmeli, A. Mor, Antibacterial properties of dermaseptin S4 derivatives with in vivo activity, Antimicrob. Agents Chemother. 46 (2002) 689–694.

[27] C. Hernandez, A. Mor, F. Dagger, P. Nicolas, A. Hernandez, E.L. Benedetti, I. Dunia, Functional and structural damage in *Leishmania mexicana* exposed to the cationic peptide dermaseptin, Eur. J. Cell. Biol. 59 (1992) 414–424.

[28] L. Efron, A. Dagan, L. Gaidukov, H. Ginsburg, A. Mor, Direct interaction of dermaseptin S4 aminoheptanoyl derivative with intraerythrocytic malaria parasite leading to increased specific antiparasitic activity in culture, J. Biol. Chem. 277 (2002) 24067–24072.

[29] G.D. Brand, J.R. Leite, L.P. Silva, S. Albuquerque, M.V. Prates, R.B. Azevedo, V. Carregaro, J.S. Silva, V.C. Sa, R.A. Brandao, C. Bloch Jr, Dermaseptins from *Phyllomedusa oreades* and *Phyllomedusa distincta*. Anti-*Trypanosoma cruzi* activity without cytotoxicity to mammalian cells, J. Biol. Chem. 277 (2002) 49332–49340.

[30] C. Lorin, H. Saidi, A. Belaid, A. Zairi, F. Baleux, H. Hocini, L. Belec, K. Hani, F. Tangy, The anti-microbial peptide dermaseptin S4 inhibits HIV-1 infectivity in vitro, Virology 334 (2005) 264–275.

[31] Y. Shin, R.W. Moni, J.E. Lueders, J.W. Daly, Effects of the amphiphilic peptides mastoparan and adenoregulin on receptor binding, G proteins, phosphoinositide breakdown, cyclic AMP generation, and calcium influx, Cell. Mol. Neurobiol. 14 (1994) 133–157.

[32] L. Marenah, S. McClean, P.R. Flatt, D.F. Orr, C. Shaw, Y.H. Abdel-Wahab, Novel insulin-releasing peptides in the skin of *Phyllomedusa trinitatis* frog include 28 amino acid peptide from dermaseptin BIV precursor, Pancreas 29 (2004) 110–115.

[33] B. Ammar, A. Perianin, A. Mor, G. Sarfat, M. Tissot, P. Nicolas, J.P. Giroud, M. Roch-Arveiller, Dermaseptin, a peptide antibiotic, stimulates microbicidal activities of polymorphonuclear leukocytes, Biochem. Biophys. Res. Commun. 247 (1998) 870–875.

[34] Y. Pouny, D. Rapaport, A. Mor, P. Nicolas, Y. Shai, Interaction of antimicrobial dermaseptin and its fluorescently labeled analogues with phospholipid membranes, Biochemistry 31 (1992) 12416–12423.

[35] Y. Shai, Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membranelytic peptides, Biochim. Biophys. Acta 1462 (1999) 55–70.

[36] D.E. Shalev, A. Mor, I. Kustanovich, Structural consequences of carboxyamidation of dermaseptin S3, Biochemistry 41 (2002) 7312–7317.

[37] O. Lequin, F. Bruston, O. Convert, G. Chassaing, P. Nicolas, Helical structure of dermaseptin B2 in a membrane-mimetic environment, Biochemistry 42 (2003) 10311–10323.

[38] M.A. Castiglione-Morelli, P. Cristinziano, A. Pepe, P.A. Temussi, Conformation–activity relationship of a novel peptide antibiotic: structural characterization of dermaseptin DS 01 in media that mimic the membrane environment, Biopolymers 80 (2005) 688–696.

[39] C. El Amri, C. Lacombe, K. Zimmerman, A. Ladram, M. Amiche, P. Nicolas, F. Bruston, The plasticins: membrane adsorption, lipid disorders, and biological activity, Biochemistry 45 (2006) 14285–14297.

[40] C. El Amri, F. Bruston, P. Joanne, C. Lacombe, P. Nicolas, Intrinsic flexibility and structural adaptability of Plasticins membrane-damaging peptides as a strategy for functional versatility, Eur. Biophys. J. 36 (2007) 901–909.
[41] R.M. Epand, H.J. Vogel, Diversity of antimicrobial peptides and their mechanisms of action, Biochim. Biophys. Acta 1462 (1999) 11–28.
[42] J.P. Powers, R.E. Hancock, The relationship between peptide structure and antibacterial activity, Peptides 24 (2003) 1681–1691.
[43] I. Zelezetsky, A. Tossi, Alpha-helical antimicrobial peptides—using a sequence template to guide structure–activity relationship studies, Biochim. Biophys. Acta 1758 (2006) 1436–1449.
[44] Z. Oren, J.C. Lerman, G.H. Gudmundsson, B. Agerberth, Y. Shai, Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: relevance to the molecular basis for its non-cell-selective activity, Biochem. J. 341 (1999) 501–513.
[45] M.A. Lemmon, J.M. Flanagan, J.F. Hunt, B.D. Adair, B.J. Bormann, C.E. Dempsey, D.M. Engelman, Glycophorin A dimerization is driven by specific interactions between transmembranealpha-helices, J. Biol. Chem. 267 (1992) 7683–7689.
[46] F. Bruston, C. Lacombe, K. Zimmermann, C. Piesse, P. Nicolas, C. El Amri, Structural malleability of plasticins: preorganized conformations in solution and relevance for antimicrobial activity, Biopolymers 86 (2007) 42–56.
[47] M.D. Lad, F. Birembaut, L.A. Clifton, R.A. Frazier, J.R. Webster, R.J. Green, Antimicrobial peptide–lipid binding interactions and binding selectivity, Biophys. J. 92 (2007) 3575–3586.
[48] F.J. Aranda, J.A. Teruel, A. Ortiz, Interaction of a synthetic peptide corresponding to the N-terminus of canine distemper virus fusion protein with phospholipid vesicles: a biophysical study, Biochim. Biophys. Acta 1618 (2003) 51–58.
[49] T.I. Lotta, I.S. Salonen, J.A. Virtanen, K.K. Eklund, P.K. Kinnunen, Fourier transform infrared study of fully hydrated dimyristoylphosphatidylglycerol: effects of Na+ on the sn-1 and sn-3 headgroup stereoisomers, Biochemistry 27 (1988) 8158–8169.
[50] S. Noinville, F. Bruston, C. El Amri, D. Baron, P. Nicolas, Conformation, orientation, and adsorption kinetics of dermaseptin B2 onto synthetic supports at aqueous/solid interface, Biophys. J. 85 (2003) 1196–1206.
[51] D. Vanhoye, F. Bruston, C. El Amri, A. Ladram, M. Amiche, P. Nicolas, Membrane association, electrostatic sequestration, and cytotoxicity of Gly-Leu-rich peptide orthologs with differing functions, Biochemistry 43 (2004) 8391–8409.
[52] A.H. Thompson, A.J. Bjourson, D.F. Orr, C. Shaw, S. McClean, A combined mass spectrometric and cDNA sequencing approach to the isolation and characterization of novel antimicrobial peptides from the skin secretions of *Phyllomedusa hypochondrialis* azurea, Peptides 28 (2007) 1331–1343.
[53] T. Isaacson, A. Soto, S. Iwamuro, F.C. Knoop, J.M. Conlon, Antimicrobial peptides with atypical structural features from the skin of the Japanese brown frog *Rana japonica*, Peptides 23 (2002) 419–425.
[54] M.L. Mangoni, N. Grovale, A. Giorgi, G. Mignogna, M. Simmaco, D. Barra, Structure–function relationships in bombinins H, antimicrobial peptides from Bombina skin secretions, Peptides 21 (2000) 1673–1679.
[55] N. Papo, Y. Shai, Can we predict biological activity of antimicrobial peptides from their interactions with model phospholipid membranes? Peptides 24 (2003) 1693–1703.
[56] D. Barra, M. Simmaco, H.G. Boman, Gene-encoded peptide antibiotics and innate immunity: do animacules have defense budget? FEBS Lett. 430 (1998) 130–134.
[57] L.P. Liu, C.M. Deber, Guidelines for membrane protein engineering derived from de novo designed model peptides, Biopolymers 47 (1998) 41–62.
[58] M. Stark, L.P. Liu, C.M. Deber, Cationic hydrophobic peptides with antimicrobial activity, Antimicrob, Agents Chemother. 46 (2002) 3585–3590.
[59] M. Fandrich, On the structural definition of amyloid fibrils and other polypeptide aggregates, Cell. Mol. Life Sci. 64 (2007) 2066–2078.
[60] M.R. Nilsson, Techniques to study amyloid fibril formation in vitro. Techniques to study amyloid fibril formation in vitro, Methods 34 (2004) 151–160.
[61] C. Auvynet, C. El Amri, C. Lacombe, F. Bruston, J. Bourdais, P. Nicolas, Y. Rosenstein, Structural requirements for antimicrobial versus chemoattractant activities for dermaseptin S9, FEBS J. 275 (2008) 4134–4151.
[62] L. Dumery, F. Bourdel, Y. Soussan, A. Fialkowsky, S. Viale, P. Nicolas, M. Reboud-Ravaux, Beta-amyloid protein aggregation: its implication in the physiopathology of Alzheimer's disease, Pathologie Biologie 49 (2001) 72–85.
[63] M.L. Waters, Aromatic interactions in peptides: impact on structure and function, Biopolymers 76 (2004) 435–445.
[64] D. Zanuy, Y. Porat, E. Gazit, R. Nussinov, A synthetic peptide derived from human immunodeficiency virus type 1 gp 120 downregulates the expression and function of chemokine receptor CCR5 and CXCR4 in monocytes by activating the 7-transmembrane G-protein-coupled receptor FPRL1/LXA4R, Structure 12 (2004) 439–455.
[65] L. Tjernberg, W. Hosia, N. Bark, J. Thyberg, J. Johansson, Charge attraction and beta propensity are necessary for amyloid fibril formation from tetrapeptides, J. Biol. Chem. 277 (2002) 43243–43246.
[66] O. Crescenzi, S. Tomaselli, R. Guerrini, S. Salvadori, A.M. D'Ursi, P.A. Temussi, D. Picone, Solution structure of the Alzheimer amyloid beta-peptide (1–42) in an apolar microenvironment. Similarity with a virus fusion domain, Eur. J. Biochem. 269 (2002) 5642–5648.
[67] O. Lequin, A. Ladram, L. Chabbert, F. Bruston, O. Convert, D. Vanhoye, G. Chassaing, P. Nicolas, M. Amiche, Dermaseptin S9, an alpha-helical antimicrobial peptide with a hydrophobic core and cationic termini, Biochemistry 45 (2006) 468–480.
[68] Y. Le, W. Gong, H.L. Tiffany, A. Tumanov, S. Nedospasov, W. Shen, N.M. Dunlop, J.L. Gao, P.M. Murphy, J.J. Oppenheim, J.M. Wang, Amyloid (beta) 42 activates a G-protein-coupled chemoattractant receptor, FPR-like-1, J. Neurosci. 21 (2001) RC123.
[69] Q. Chen, D. Wade, K. Kurosaka, Z.Y. Wang, J.J. Oppenheim, D. Yang, Temporin A and related frog antimicrobial peptides use formyl peptide receptor-like 1 as a receptor to chemoattract phagocytes, J. Immunol. 173 (2004) 2652–2659.
[70] H.A. Lashuel, P.T. Lansbury Jr, Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins, Q. Rev. Biophys. 39 (2006) 167–201.
[71] M. Del Mar Martinez-Senac, J. Villalain, J.C. Gomez-Fernandez, Structure of the Alzheimer beta-amyloid peptide (25–35) and its interaction with negatively charged phospholipid vesicles, Eur. J. Biochem. 265 (1999) 744–753.
[72] R. Sood, Domanov, P.K. Kinnunen, Fluorescent temporin B derivative and its binding to liposomes, J. Fluoresc. 17 (2007) 223–234.
[73] H. Zhao, R. Sood, A. Jutila, S. Bose, G. Fimland, J. Nissen-Meyer, P.K. Kinnunen, Interaction of the antimicrobial peptide pheromone Plantaricin A with model membranes: implications for a novel mechanism of action, Biochim. Biophys. Acta 1758 (2006) 1461–1474.
[74] H. Zhao, A. Jutila, T. Nurminen, S.A. Wickstrom, J. Keski-Oja, P.K. Kinnunen, P. K. Binding of endostatin to phosphatidylserine-containing membranes and formation of amyloid-like fibers, Biochemistry 44 (2005) 2857–2863.
[75] Y. Shai, Molecular recognition between membrane-spanning polypeptides, Trends Biochem. Sci. 20 (1995) 460–464.
[76] B.L. Sollod, D. Wilson, O. Zhaxybayeva, J.P. Gogarten, R. Drinkwater, G.F. King, Were arachnids the first to use combinatorial peptide libraries? Peptides 26 (2005) 131–139.
[77] R.S. Norton, B.M. Olivera, Conotoxins down under, Toxicon 48 (2006) 780–798.
[78] B.M. Olivera, Conus venom peptides: reflections from the biology of clades and species, Annu. Rev. Ecol. Syst. 33 (2002) 25–47.
[79] L.D. Possani, B. Becerril, M. Delepierre, J. Tytgat, Scorpion toxins specific for Na+-channels, Eur. J. Biochem. 264 (1999) 287–300.
[80] B.M. Olivera, Conus peptides: biodiversity-based discovery and exogenomics, J. Biol. Chem. 281 (2006) 31173–31177.
[81] A. Mor, M. Amiche, P. Nicolas, Structure, synthesis, and activity of dermaseptin B, a novel vertebrate defensive peptide from frog skin: relationship with adenoregulin, Biochemistry 33 (1994) 6642–6650.
[82] S. Charpentier, M. Amiche, J. Mester, V. Vouille, J.P. Le Caer, P. Nicolas, A. Delfour, Structure, synthesis, and molecular cloning of dermaseptins B, a family of skin peptide antibiotics, J. Biol. Chem. 273 (1998) 14690–14697.
[83] T. Chen, L. Tang, C. Shaw, Identification of three novel *Phyllomedusa sauvagei* dermaseptins (sVI–sVIII) by cloning from a skin secretion-derived cDNA library, Regul. Pept. 116 (2003) 139–146.
[84] C. Wechselberger, Cloning of cDNAs encoding new peptides of the dermaseptin-family, Biochim. Biophys. Acta 1388 (1998) 279–283.
[85] L. Marenah, C. Shaw, D.F. Orr, S. McClean, P.R. Flatt, Y.H. Abdel-Wahab, Isolation and characterisation of an unexpected class of insulinotropic peptides in the skin of the frog *Agalychnis litodryas*, Regul. Pept. 120 (2004) 33–38.
[86] T. Chen, M. Zhou, R. Gagliardo, B. Walker, C. Shaw, Elements of the granular gland peptidome and transcriptome persist in air-dried skin of the South American orange-legged leaf frog, *Phyllomedusa hypocondrialis*, Peptides 27 (2006) 2129–2136.
[87] G.D. Brand, J.R. Leite, S.M. de Sá Mandel, D.A. Mesquita, L.P. Silva, M.V. Prates, E.A. Barbosa, F. Vinecky, G.R. Martins, J.H. Galasso, S.A. Kuckelhaus, R.N. Sampaio, J.R. Furtado Jr, A.C. Andrade, C. Bloch Jr, Novel dermaseptins from *Phyllomedusa hypochondrialis* (Amphibia), Biochem. Biophys. Res. Commun. 347 (2006) 739–746.
[88] K. Conceição, K. Konno, M. Richardson, M.M. Antoniazzi, C. Jared, S. Daffre, A.C. Camargo, D.C. Pimenta, Isolation and biochemical characterization of peptides presenting antimicrobial activity from the skin of *Phyllomedusa hypochondrialis*, Peptides 27 (2006) 3092–3099.
[89] C.V. Batista, L.R. da Silva, A. Sebben, A. Scaloni, L. Ferrara, G.R. Paiva, T. Olamendi-Portugal, L.D. Possani, C. Bloch Jr, Antimicrobial peptides from the Brazilian frog *Phyllomedusa distincta*, Peptides 20 (1999) 679–686.
[90] J.M. Conlon, D.C. Woodhams, H. Raza, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, L.A. Rollins-Smith, Peptides with differential cytolytic activity from skin secretions of the lemur leaf frog *Hylomantis lemur* (Hylidae: Phyllomedusinae), Toxicon 50 (2007) 498–506.
[91] L.R. Silva, C.V.F. Batista, M.V. Prates, M. Gordo, C. Bloch Jr., A new antimicrobial peptide homologous to the dermaseptins isolated from *Phyllomedusa tarsius*, Toxicon 38 (2000) Abstract 487595.
[92] R. Leite, L.P. Silva, M.I. Rodrigues, M.V. Prates, G.D. Brand, B.M. Lacava, R.B. Azevedo, A.L. Bocca, S. Albuquerque, C. Bloch Jr., Phylloseptins: a novel class of anti-bacterial and anti-protozoan peptides from the *Phyllomedusa genus*, Peptides 26 (2005) 565–573.
[93] M. Amiche, A.A. Seon, H. Wroblewski, P. Nicolas, Isolation of dermatoxin from frog skin, an antibacterial peptide encoded by a novel member of the dermaseptin genes family, Eur. J. Biochem. 267 (2000) 4583–4592.
[94] T. Chen, B. Walker, M. Zhou, C. Shaw, Dermatoxin and phylloxin from the waxy monkey frog, *Phyllomedusa sauvagei*: cloning of precursor cDNAs and structural characterization from lyophilized skin secretion, Regul. Pept. 129 (2005) 103–108.
[95] T.N. Pierre, A.A. Seon, M. Amiche, P. Nicolas, Phylloxin, a novel peptide antibiotic of the dermaseptin family of antimicrobial/opioid peptide precursors, Eur. J. Biochem. 267 (2000) 370–378.
[96] A.H. Thompson, A.J. Bjourson, D.F. Orr, C. Shaw, S. McClean, Amphibian skin secretomics: application of parallel quadrupole time-of-flight mass spectrometry and peptide precursor cDNA cloning to rapidly characterize the skin secretory peptidome of *Phyllomedusa hypochondrialis* azurea: discovery of a novel peptide family, the hyposins, J. Proteome Res. 6 (2007) 3604–3613.
[97] C.V. Batista, A. Scaloni, D.J. Rigden, L.R. Silva, A. Rodrigues Romero, R. Dukor, A. Sebben, F. Talamo, C. Bloch, A novel heterodimeric antimicrobial peptide from the tree frog *Phyllomedusa distincta*, FEBS Lett. 494 (2001) 85–89.