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REVIEW

Intrinsic flexibility and structural adaptability of Plasticins membrane-damaging peptides as a strategy for functional versatility

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Abstract The Plasticins are a family of antimicrobial, 23–29-residue Gly-Leu-rich ortholog peptides from the frog skin that have very similar amino acid sequences, hydrophobicities, and amphipathicities but differ markedly in their conformational plasticity and spectrum of activity. The intrinsic flexibility and structural malleability of Plasticins modulate their ability to bind to and disrupt the bilayer membranes of prokaryotic and eukaryotic cells, and/or to reach intracellular targets, therefore, triggering functional versatility. The discussion is opened herein on several examples of other membrane-active peptides, like viral fusion peptides, cell-penetrating peptides, that are able to display antimicrobial activity. Hence, Plasticins could be regarded as models of multipotent membrane-active peptides guided by structural plasticity.

Keywords Plasticins · Dermaseptins · Structural polymorphism · Conformational flexibility · Antimicrobial peptide · Frog skin

Introduction

The dermaseptins are a superfamily of gene-encoded host-defence peptides that are produced by the skin of Hylidae frogs (Nicolas et al. 2003). These peptides are genetically related, with a remarkable identity in signal sequences and acidic propieces of their biosynthetic polypeptide precursors, but have clearly evolved and diverged into several families of peptides that display great diversity in antimicrobial/cytotoxic activities and specificities by fixing amino acid substitutions with high frequency (Amiche et al. 1999; Duda et al. 2002; Nicolas et al. 2003). Most of these peptides, including the dermaseptins S and B (Charpentier et al. 1998; Mor et al. 1991), the dermatoxins (Amiche et al. 2000), the phylloxins (Pierre et al. 2000), and the phylloseptins (Leite et al. 2005) are cationic because of their lysine content, and form an amphipathic α-helix when bound to anionic/zwitterionic lipid bilayers, with their polar and apolar amino acids on opposing surfaces along the long axis of the helix (Noinville et al. 2003; Shai 1999). These structural elements are believed to play a crucial role in the binding of these peptides to the negatively charged outer leaflet of bacterial bilayers. Once bound, the hydrophobic face of the helix permits the peptide to enter the membrane interior, thereby, triggering membrane curvature, local fusion of the membrane leaflets, pore formation, cracks, and membrane disruption (Bechinger and Lohner 2006; Papo and Shai 2003; Rinaldi 2002; Shai 1999). Similar behaviours are exhibited by other extensively studied linear helical antimicrobial peptides like bee venom toxin melittin (Bechinger and Lohner 2006; Huang 2006), insect cecropin P1 (Brogden et al. 2003), frog peptide magainin (Bechinger and Lohner 2006; Wieprecht et al. 1997), and human cathelicidin LL-37 (Durr et al. 2006). Although this can provide microbial specificity, there is no simple correlation between the degree of helical structure, the peptide charge, and the antimicrobial potency or the toxicity for eukaryotic cells (Zelezetsky et al. 2005). In another hand, linear derivatives of mammalian disulfide-bridged antimicrobial peptides with β-sheet structure display high conformational flexibility and still present antimicrobial activity (Fazio et al. 2007;

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Rao 1999). This raises the question of the other parameters involved in membrane-binding and membrane-damaging properties of linear antimicrobial peptides (Zelezetsky et al. 2005).

Among peptides of the dermaseptin superfamily, the Gly-Leu-rich peptides, revealed by evolutive history reconstruction from conserved dermaseptin preprosequence transcript, form a well-demarcated family (Vanhoye et al. 2003). This family includes the neutral peptides DRP-AA 2–5 (from *Agalychnis annae*), DRP-AC1 and 2 (from *Agalychnis callidryas*), DRP-PD 3–6 (from *Pachymedusa dacnicolor*), and the cationic peptide DRP-PBN2 (from *Phyllomedusa bicolor*). These 23–29-residue peptides are rich in glycine and leucine (29 and 25% on average) and contain an extended glycine-zipper motif GXXXGXX XGXXXG (where X is any residue). This motif is strongly overrepresented in membrane protein sequences and plays a crucial role in promoting the formation of oligomeric helical bundles (Kim et al. 2005). Contrasting with amphipathic helical dermaseptins, Gly-Leu-rich peptides were found to display considerable structural polymorphism leading to functional versatility, hence the name Plasticins given to this peptide family by El Amri et al. (El Amri et al. 2006). The most studied Plasticins (Table 1) have very similar amino acid sequences (13 invariant residues), hydrophobicities and amphipathicities**,** but differ markedly in their activity spectra (Vanhoye et al. 2004). Phylogenetic reconstruction and synthesis of the ancestral peptide sequence ANC and peptide analogs with altered net charges, [$K^{8,12}$]-ANC, [$K^{8,12}$]-DRP-PD 3–6, and [$K^{8,12}$, F^{18}]-DRP-PD 3–6, were conducted. Positive selection operated at a molecular level in the early stage of frog species divergence producing a novel antimicrobial function from an inactive ancestral peptide, and that lysine residues were selected (Duda et al. 2002; Vanhoye et al. 2004, 2003). This series of functionally divergent but closely related Gly-rich-peptides constitutes a good model to address the relationships between structural polymorphism, membrane-interacting property and biological activity.

This minireview summarizes recent advances in identifying peptide parameters (intrinsic flexibility, presculpted conformational landscape, competing conformational states and molecular adaptability) that may be responsible for variability in mechanisms of action and antimicrobial versus cytotoxic specificities. We also open the discussion on functional versatility of Plasticins through examples of multipotent membrane-active peptides. We propose that intrinsic structural plasticity of Plasticins modulated by lipid microenvironments may be a major guide for induction of a given biological effect.

Conformation of membrane-bound plasticins

It is becoming increasingly clear that perturbation by antimicrobial peptides of the membrane near hydrophobic-hydrophilic interface leads to a thinning of the lipid bilayer that precedes loss of permeability barrier (Sato and Feix 2006). The degree of perturbation is thus in part linked to peptide shape. Table 2 summarized Plasticins conformational features including also their capacity to undergo structural interconversions at membrane interface as revealed by molecular dynamics simulations and FTIR spectroscopies. Despite very similar amino acid sequences, Plasticins adopted various structural fittings at anionic and zwitterionic membrane interfaces including helices, destabilized helix states, β-hairpin, β-sheet and disordered states. Cationic Plasticins are mainly helical when bound to anionic DMPG or zwitterionic DMPC phospholipid vesicles, but the contribution of β-sheet structures increases when mixed to zwitterionic vesicles. DRP-PD 3–6, which has no net charge, adopts a helical structure when bound to anionic vesicles but is predominantly β-sheeted in the presence of zwitterionic phospholipids. In contrast, ANC, the other neutral plasticin, displays predominant β-structures with both types of vesicles. CD spectroscopy and NH/ND-exchange kinetics obtained by ATR-FTIR showed that neutral Plasticins self-associate when bound to DMPG (DRP-PD 3–6 and ANC) or DMPC (ANC) vesicles. Molecular dynamics simulations performed in apolar medium ($\varepsilon = 5$) on the six Plasticins through one ns, starting from an ideal helix, demonstrated differences in the structural malleability

Table 1 Primary structures of Plasticins

DRP-PBN2	GLVTSLIKGAGKLLGGLFGSVTGa
[$K^{8,12}$]- ANC	GLVTGLLKTAGKLLGDLFGSLTGa
[$K^{8,12}$, F^{18}]- DRP-PD 3-6	GVVTDLLKTAGKLLGNLFGSLSGa
[$K^{8,12}$]- DRP-PD 3-6	GVVTDLLKTAGKLLGNLVGSLSGa
DRP-PD 3-6	GVVTDLLNTAGGLLGNLVGSLSGa
ANC	GLVTGLLNTAGGLLGDLFGSLTGa

Cationic Plasticins

Neutral Plasticins

Identical amino acids have shaded background**.** The **GXXXGXXXGXXXG** glycine-zippers and **GXXXG** motifs are underlined; a = amide

Table 2 Conformation of Plasticins in the presence of DMPC or DMPG vesicles as assessed by FTIR spectroscopies, and conformational switch propensity at membrane interfaces obtained from MDS (adapted from El Amri et al. 2006)

	DMPC		DMPG		Conformational switch propensity
	α-Helix (%)	β-Structure (%)	α-Helix (%)	β-Structure (%)	
DRP-PBN2	47	27	65	0	α → helix disturbed
DRP-PD 3–6	16	62	64	9	α → random coil → β-disturbed
[$K^{8,12}$]-DRP-PD 3–6	51	35	68	4	α → random coil
[$K^{8,12}$, F^{18}]-DRP-PD 3–6	60	30	63	18	α → β-hairpin
ANC	30	50	25	39	α → random coil → β-hairpin/β-turn
[$K^{8,12}$]- ANC	38	36	54	17	α → random coil

at membrane-mimetic interfaces (Table 2). Two behaviours are observed: neutral Plasticins (DRP-PD3–6 and ANC) underwent transitions between three conformations (helix/coil/β-structure), while cationic plasticins underwent various transitions between two states. DRP-PBN2 oscillated between helix and disturbed helix, while the two analogs [$K^{8,12}$]-DRP-PD 3–6 and [$K^{8,12}$]-ANC underwent classical helix-coil transition, the third analog [$K^{8,12}$, F^{18}]-DRP-PD 3–6 was found to be the most foldable within β-hairpin related structures. 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 (El Amri et al. 2006).

It has often been reported that antimicrobial potency is associated with an amphipathic structure, being either an α-helix or a β-sheet (Epand and Vogel 1999; Powers and Hancock 2003; Zelezetsky and Tossi 2006). Figure 1 illustrates the repartition of hydrophobic and hydrophilic residues of the six more studied plasticins in three different structural states, i.e., helix, β-strand, and β-hairpin. While all these peptides 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. Some designed linear cationic peptides with high amphipathic β-sheet potentials were found to weakly interact with PC-containing membranes and have a high antimicrobial potential. These peptides may thus be promising candidates for antimicrobial agents, especially for topical applications, with good selectivity between bacterial and mammalian cells (Doherty et al. 2006; Jin et al. 2005). In summary, membrane-bound cationic Plasticins exhibit antimicrobial activity when displaying an α-helix/β-structure ratio ≥ 1. In contrast, neutral Plasticins have an α-helix/β-structure ratio <1 when bound to phospholipid membranes, and are devoid of antimicrobial activity.

Pre-organized structure of Plasticins in aqueous solution: β-hairpin as conformational lock or template

The intrinsic conformational preferences of Plasticins in aqueous solutions have been investigated to probe to what extent pre-existent peptide conformations (intrinsic conformational landscape) may be responsible for variability in bioactive, membrane-bound, conformations and membrane-disturbing properties (Bruston et al. 2007). DRP-PBN2 and [$K^{8,12}$]-DRP-PD 3–6 displayed mixture of unordered and β-turn structures in aqueous media, while neutral peptides displayed high contents of aggregated β-structures and turns. [$K^{8,12}$, F^{18}]-DRP-PD 3–6 distinguished by its high content of β-hairpin structure together with low amounts of β-turns and unordered structure. It is known that the amino acid sequence of a turn can dictate not only the hairpin stability and turn conformation, but also the register of interstrand H-bond interactions (Ramirez-Alvarado et al. 1997). Although turn regions are early formed for all Plasticins, they do not always result in an overall folding within β-hairpin. The residue at position eight plays a major role in initiating the folding, while position 12 seemed not to be critical. The contribution of hydrophobic side chain to the initiation and stabilization of the hairpin structure is demonstrated from peptides encompassing a phenylalanine residue at position 18 ([$K^{8,12}$, F^{18}]-DRP-PD 3–6, [$K^{8,12}$]-ANC and DRP-PBN2). In addition, the presence inside the turn region of the β-strand promoting residue threonine in all Plasticins except DRP-PBN2 is also a key factor acting as a hinge and a hydrophobic stick ensuring cohesion of the loopy region. Conformational preferences and stability of Plasticins do not exert a profound influence on the antimicrobial potency, and there is no simple correlation between structural flexibility versus rigidity and bioactivity. For instance, increased antimicrobial potency may not always result from rigid conformational landscapes. This was already suggested by Gellman and co-workers who examined the role of structural rigidity in antimicrobial activity of helix-forming oligomers of β-amino acids (Raguse et al.

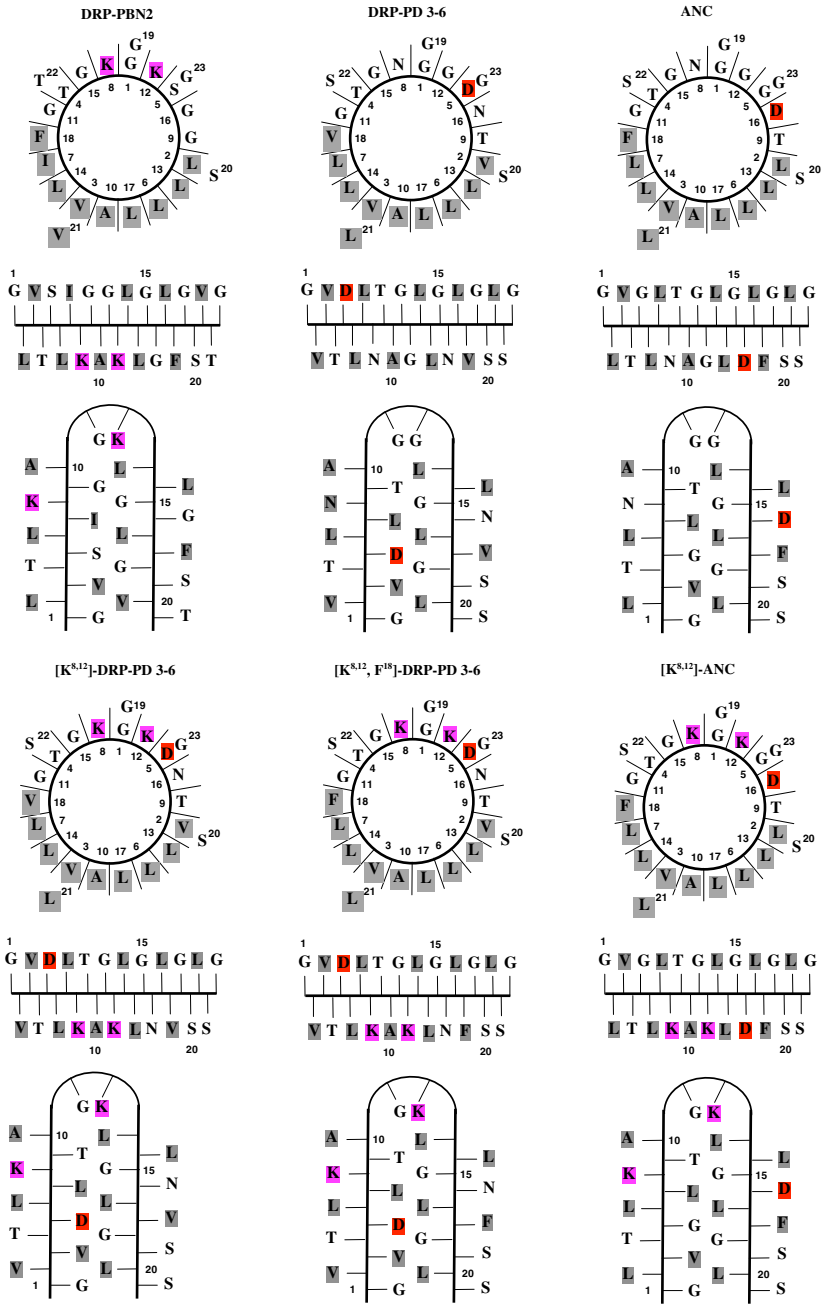

Fig. 1 Helical wheel, β-strand and β-hairpin diagrams showing the distribution of amino acid side chains for Plasticins. Hydrophobic (*gray background*), positively charged (*blue background*), and negatively charged (*red background*) side chains are highlighted

2002). However, β-hairpin foldability and stability may explain subtle differences between the antimicrobial activities and mechanisms of action of the most potent cationic plasticins DRP-PBN2 and [$K^{8,12}$, F^{18}]-DRP-PD 3–6. β-hairpin preformed in solution may act as a conformational lock that prevents switch to α-helical structure, thus lowering antimicrobial efficiency. In contrast, β-hairpin-shaped conformations could also serve as a template for the formation of helical structures at the membrane interface, facilitating conformational switch during membrane fusion process as reported for the HIV-1 Gp41 amino-terminal fusion peptide (Lorizate et al. 2006; Reichert et al. 2007).

Plasticity of molecular interactions

In addition to amphipathicity and cationicity, the plasticity of molecular interactions between membranotropic peptides and biomembranes (Nagpal et al. 2002) is an emerging fact that may allow a better understanding of antimicrobial peptide mechanisms of action.

Binding of Plasticins to lipid bilayers by SPR. The affinity of antimicrobial peptides for phospholipid bilayers is a critical factor influencing their selectivity and potency (Jelinek and Kolusheva 2005; Papo and Shai 2003; Shai 1999). Cationic Plasticins bind to anionic membranes in a 2-step process. In a first step, the cationic peptide binds to the membrane surface because of the hydrophobic effect and several non-specific interactions. In a second step, the subsequent formation and adjustment of the peptide helix result in tightly bound peptide-membrane complexes that are primarily stabilized by the peptide aliphatic and aromatic residues (Vanhoye et al. 2004). Peptides encompassing phenylalanine residue formed more dense PL* complexes with DMPG bilayer than did [$K^{8,12}$]-DRP-PD 3–6 (Table 3). The presence of aromatic residue clearly prolonged the contact time between peptide and membrane (El Amri et al. 2006), allowing further structural rearrangements (Stahelin and Cho 2001). This behaviour is significantly different from other small membrane-damaging cationic linear peptides such as magainins, cecropins and dermaseptins (Sato and Feix 2006). These latter peptides, having low hydrophobicity and conformational flexibility, interact with anionic lipids ~100-times more than with zwitterionic lipids. However, this difference in binding is mainly due to the first binding step that is governed by non-specific long-range electrostatic interactions between the peptide lysine residues and the anionic headgroups of the phospholipids. Plasticin adsorption densities at the equilibrium or after 10 min of desorption (PL* complexes) were found to be insignificant at concentrations below the MIC. Adsorption densities were primary determined using hydrophobic synthetic sensor chip (HPA) (El Amri et al. 2006). This quantification provided information on the structure of irreversibly bound peptides and particularly underlined adsorption of various structural species. Moreover, cationic Plasticins formed multilayers when adsorbed onto either DMPC or DMPG phospholipids but, only DMPC headgroups partially prevented their anchorage, except for ancestral analog ([$K^{8,12}$]-ANC). Their α-helix content is always greater than the β-structure content. In contrast, the adsorption densities of neutral Plasticins on DMPC or DMPG are about 10 times lower than those of cationic Plasticins, due to their own self-association (El Amri et al. 2006).

Membrane damaging activity and membrane structure disturbance. Plasticins vary substantially in their abilities to depolarize the cytoplasmic membrane of bacterial cells

Table 3 Adsorption densities of Plasticins investigated by SPR and expressed as ng per mm^2

(A)	Hydrophobic surface (ng mm^{-2})	DMPC (ng mm^{-2})	DMPG (ng mm^{-2})
DRP-PBN2	0.7	1.2	2.8
DRP-PD 3–6	0.5	0.2	0.1
[$K^{8,12}$]- DRP-PD 3–6	0.4	4.2	3.0
[$K^{8,12}$, F^{18}]- DRP-PD 3–6	0.7	3.7	3.5
ANC	0.5	0.2	0.2
[$K^{8,12}$]- ANC	0.7	1.7	3.3

(B) P + L ↔ PL ↔ PL*	DMPC[a] (ng mm^{-2})	DMPG[a] (ng mm^{-2})
DRP-PBN2	0.3	1.2
DRP-PD 3–6	0.1	ND
[$K^{8,12}$]- DRP-PD 3–6	0.2	1.0
[$K^{8,12}$, F^{18}]- DRP-PD 3–6	0.3	1.2
ANC	ND	ND
[$K^{8,12}$]- ANC	0.5	1.2

[a] The PL* complex density was evaluated from the SPR response after 10 min of desorption

A hydrophobic surface (HPA) and bilayers of DMPG and DMPC phospholipids. B/PL* complex density (adapted from El Amri et al. 2006). If the peptide molecule is considered to be a perfect α-helix spanning residues 1–23, its contact surface should be a rectangle measuring 3.5 nm long by 1.5 nm wide, giving a surface area of 5 nm^2 . A peptide that adopts an extended or a β-hairpin structure will have a contact surface area of 10–12 nm^2 . Consequently, a hydrophobic sensor chip surface such as HPA can adsorb 0.9 ng of helical peptide and only 0.4 ng of a peptide having an extended or an hairpin structure

Table 4 Membrane-damaging activities of Plasticins

A/DMPG

	Antimicrobial activity	Membrane potential	Disturbance at membrane interface (%)	Disturbance of bilayer core (%)
DRP-PBN2	Bactericidal	++	23	5
DRP-PD 3–6	ND	ND	17	4
[$K^{8,12}$]-DRP-PD 3–6	Bacteriostatic	ND	17	3
[$K^{8,12}$, F^{18}]-DRP-PD 3–6	Bacteriostatic	+++	23	3
ANC	ND	ND	17	5
[$K^{8,12}$]-ANC	Bacteriostatic	+	17	6

B/DMPC

	Cytotoxicity	Disturbance at membrane interface (%)	Disturbance of bilayer core (%)
DRP-PBN2	+++	13	4
DRP-PD 3–6	ND	0	0
[$K^{8,12}$]-DRP-PD 3–6	ND	0	3
[$K^{8,12}$, F^{18}]-DRP-PD 3–6	++	7	2
ANC	ND	0	0
[$K^{8,12}$]-ANC	+	7	4

ND not determined

(Table 4). No correlation between membrane permeabilization and antibacterial activity is noticed. For example, DRP-PBN2 exhibits bactericidal activity against *E. coli* but has a reduced ability to disrupt the bacterial membrane potential (El Amri et al. 2006). In contrast, [$K^{8,12}$, F^{18}]-DRP-PD 3–6, which folds within a β-hairpin shaped structure displays the highest potency to dissipate the membrane potential but only exerts bacteriostatic effects. This peptide appears to act *via* membrane depolarization. Other bacteriostatic Plasticins such as [$K^{8,12}$]-ANC have no effect on the membrane potential of the target cells.

FTIR experiments with DMPG or DMPC allowed to measure disturbances of membrane assembly at the interface (Blume et al. 1988; Castano et al. 1999; Lotta et al. 1988) and/or bilayer alkyl chains (Cameron et al. 1980) (Table 4a and b). CO ester 1727/1742 ratio percentage, indicative of a disturbance at membrane interface, increase consecutive to the binding of the peptides is more pronounced with anionic membrane than with zwitterionic membrane. Binding of cationic Plasticins to DMPG vesicles (Table 4a) illustrates membrane dehydration and formation of peptide-membrane hydrogen bonds. In contrast, the binding of the peptides to DMPC vesicles shows their reduced ability to dehydrate the membrane headgroups and to form hydrogen bonds (Table 4b).

Cationic Plasticins bearing phenylalanine (DRP-PBN2, [$K^{8,12}$]-ANC and [$K^{8,12}$, F^{18}]-PD 3–6), induce redistribution of the band components in DMPC vesicles, with higher 1727/1742 ratios compared to pure DMPC (Table 4b). This could reflect more water molecules associated to the ester groups due to greater peptide partition into the interface region (El Amri et al. 2006).

All the Plasticins, exclusively in the presence of DMPG, cause an increase in the methylene stretching frequencies ($\nu_{AS}(CH_2)$), broadening of the corresponding absorbance bands, and a decrease in the alkyl chain order parameter underlying transition of lipids from the well-ordered gel state to the more disordered liquid-crystalline state (Table 4a, b). This result was also observed for Lactocin705β in presence of DPPC (Castellano et al. 2007).

Neutral plasticins (DRP-PD 3–6 and ANC) interact weakly with DMPG vesicles, causing little perturbations at the membrane interface but inducing significant disturbances in the membrane interior. They interact with DMPC vesicles without perturbing either the interface or the bilayer interior. During bacterial attack, many peptides can be retrieved in the frog skin secretions. The resulting cocktail contains simultaneously neutral, anionic and cationic peptides. Inactive neutral peptides enhanced the activity of cationic peptides. Thus, neutral peptides could act as primary membrane disturbing peptides by adopting a transmembrane orientation in synergy with cationic peptides (Vanhoye et al. 2004).

Oligomerization stage sequentiality. Selectivity and activity of antimicrobial peptides may additionally depend on properties other than their lipid-binding characteristics. For instance, peptides have to cross the bacterial cell wall before reaching the cytoplasmic membrane, a process that strongly depends on the structure and oligomeric state of the peptides. For instance, human cathelicidin LL-37 which disrupt membranes via a carpet mechanism, approaches the membrane in an α-helical oligomeric state (Oren et al. 1999). The sequence of plasticins encompasses three GXXXG motifs, known to mediate interactions between helical transmembrane domains of membrane proteins or helical fusion peptides (Kim et al. 2005). Indeed, Plasticins in the presence of crosslinker and incubated in various mimetic membrane media were shown to be mainly monomeric by SDS-PAGE analysis. Moreover, measurement of their translational diffusion coefficients by pulse field gradient-NMR (PFG-NMR) reveals the existence of monomer-dimer equilibrium (unpublished data). Thus, the presence of GXXXG motifs is not sufficient to promote oligomerization. Other factors may to be involved such as the sequence context and the intrinsic conformational flexibility. It is thus probable that the differences in biological activity of Plasticins are related to shifts of classical autoassociation stage, i.e. differences in the sequentiality of peptide oligomerization.

Flexible linear antimicrobial peptides

It is generally assumed that linear amphipathic antimicrobial peptides operate through random coil-to-helix structure transition upon interaction with microbial membrane (Ladokhin and White 1999). However, a growing number of studies have convincingly demonstrated that structural versatility of the peptides in the vicinity of membranes may lead to alternative mechanisms of action. Clavanin constitutes an interesting example where conformational flexibility, mainly driven by glycines, is a major determinant for antimicrobial activity (van Kan et al. 2001). Class II bacteriocins, including brochocins, thermophilins, plantaricins or lactococcins are also glycine-rich, cationic, and hydrophobic peptides, which display conformational plasticity as Plasticins. Numerous works have demonstrated, that membrane disruption models elaborated for amphiphilic peptides (e.g., toroidal pore or carpet model) cannot adequately describe the bactericidal action of bacteriocins. Specific targets seem rather to be involved in pore formation and other activities (Nes and Holo 2000). Recently, Wimmer et al. have emphasized the versatility of novispirin G-10 interactions, a 18-residue designed cationic peptide derived from the N-terminal part of a sheep antimicrobial peptide, with detergent and lipids (Wimmer et al. 2006). Novispirin ability to bind to these

Table 5 Structural interconversions from aqueous to membrane environment and plurifunctionality of several representative membrane-active peptides

	Conformational transitions from aqueous to membrane-like environments	Biological activities
Plasticins (Bruston et al. 2007; El Amri et al. 2006; Vanhoye et al. 2004) **DRP-PBN2 :** GLVTSLI**K**GAG**K**LLGGL**F**GSVTGa **[$K^{8,12}$, F^{18}]- DRP-PD 3-6 :** GVVTDLL**K**TAG**K**LLGNL**F**GSLSGa	**unordered structures + β-turns/β-hairpins/aggregatedβ-structures** ↓ **aggregatedβ-structures/β-turns/α-helical**	Antimicrobial Proapoptotic Fusogenic?
Indolicidin (Chan et al. 2006; Hsu et al. 2005; Robinson et al. 1998) ILPWKPWWPWRR-NH_2	**unordered structures + β-turns** ↓ **unordered structures/β-turns/α-helical**	Antiviral (anti-HIV1) Antimicrobial Proapoptotic
Penetratins (Henriques et al. 2006; Magzoub et al. 2002) RQIKIWFQNRRMKWKK	**α-helical +unordered structures** ↓ **aggregatedβ-structures/β-turns**	Antimicrobial Cell-penetrating
Gp41 fusion peptide (Buzon et al. 2005; Cole et al. 2003;) AVGIGALFLGFLGAAGSTMGARS GALFLGAAGSTMGAWSQPKKKRKV	**α-helical +unordered structures** ↓ **aggregatedβ-structures**	Antimicrobial Fusogenic Cell-penetrating

amphiphiles and to form α-helical structure was found to be sensitive to the electrostatics environment. In summary, the antimicrobial potency of linear flexible peptides, adopting various conformational states in equilibrium or not, may be driven by functionally suitable topologies.

Towards new biological effects of membranotropic action

Biologically relevant interactions must be specific at the molecular level (Nagpal et al. 2002). Both the hydrophobic clustering and the electrostatic interactions accompanied by the relative conformational flexibility in a peptide molecule would provide certain leeway within this specificity. Table 5 points out the importance of structural interconversions for the acquisition of a given biological activity through the example of several representative membrane active peptides in comparison with Plasticins (Indolicidin, Penetratin, Gp41 fusion peptides). Plasticity of interactions could also ensure the recognition of a broad spectrum of organisms, which would be a necessity in host defence. Studies on plasticins gave insights on the many ways a peptide could perturb biomembranes, leading to a biological activity. Previous studies have revealed proapoptotic properties of two cationic Plasticins against Hela cells. Interestingly, structural and orientational plasticity is also an interesting property of cell-penetrating peptides (Clayton et al. 2006). Structural versatility of tryptophan-rich indolicidin seems also to be a key for the acquisition of variable activities (antiviral, antimicrobial and proapoptotic) (Chan et al. 2006; Hsu et al. 2005; Robinson et al. 1998). Moreover, it has been reported that peptide fusogenicity largely involves conformationally disordered peptides with a pronounced structural plasticity (Hofmann et al. 2004; Reichert et al. 2007). Plasticins could be regarded as models of multipotent membrane-active peptides guided by structural plasticity. Ongoing studies in the laboratory aim to explore their fusogenicity.

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References

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

Amiche M, Seon AA, Wroblewski H, Nicolas P (2000) Isolation of dermatoxin from frog skin, an antibacterial peptide encoded by a novel member of the dermaseptin genes family. Eur J Biochem 267:4583–4592

Bechinger B, Lohner K (2006) Detergent-like actions of linear amphipathic cationic antimicrobial peptides. Biochim Biophys Acta 1758:1529–1539

Blume A, Hubner W, Messner G (1988) Fourier transform infrared spectroscopy of 13C = O-labeled phospholipids hydrogen bonding to carbonyl groups. Biochemistry 27:8239–8249

Brogden KA, Ackermann M, McCray PB Jr, Tack BF (2003) Antimicrobial peptides in animals and their role in host defences. Int J Antimicrob Agents 22:465–478

Bruston F, Lacombe C, Zimmermann K, Piesse C, Nicolas P, El Amri C (2007) Structural malleability of plasticins: preorganized conformations in solution and relevance for antimicrobial activity. Biopolymers 86:42–56

Buzon V, Padros E, Cladera J (2005) Interaction of fusion peptides from HIV gp41 with membranes: a time-resolved membrane binding, lipid mixing, and structural study. Biochemistry 44:13354–13364

Cameron DG, Casal HL, Mantsch HH (1980) Characterization of the pretransition in 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine by Fourier transform infrared spectroscopy. Biochemistry 19:3665–3672

Castano S, Desbat B, Laguerre M, Dufourcq J (1999) Structure, orientation and affinity for interfaces and lipids of ideally amphipathic lytic LiKj(i=2j) peptides. Biochim Biophys Acta 1416:176–194

Castellano P, Vignolo G, Farias RN, Arrondo JL, Chehin R (2007) Molecular view by fourier transform infrared spectroscopy of the relationship between lactocin 705 and membranes: speculations on antimicrobial mechanism. Appl Environ Microbiol 73:415–420

Chan DI, Prenner EJ, Vogel HJ (2006) Tryptophan- and arginine-rich antimicrobial peptides: structures and mechanisms of action. Biochim Biophys Acta 1758:1184–1202

Charpentier S, Amiche M, Mester J, Vouille V, Le Caer JP, Nicolas P, Delfour A (1998) Structure, synthesis, and molecular cloning of dermaseptins B, a family of skin peptide antibiotics. J Biol Chem 273:14690–14697

Clayton AH, Atcliffe BW, Howlett GJ, Sawyer WH (2006) Conformation and orientation of penetratin in phospholipid membranes. J Pept Sci 12:233–238

Cole AM, Liao HI, Ganz T, Yang OO (2003) Antibacterial activity of peptides derived from envelope glycoproteins of HIV-1. FEBS Lett 535:195–199

Doherty T, Waring AJ, Hong M (2006) Peptide-lipid interactions of the beta-hairpin antimicrobial peptide tachyplesin and its linear derivatives from solid-state NMR. Biochim Biophys Acta 1758:1285–1291

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

Durr UH, Sudheendra US, Ramamoorthy A (2006) LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochim Biophys Acta 1758:1408–1425

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

Epand RM, Vogel HJ (1999) Diversity of antimicrobial peptides and their mechanisms of action. Biochim Biophys Acta 1462:11–28

Fazio MA, Jouvensal L, Vovelle F, Bulet P, Miranda MT, Daffre S, Miranda A (2007) Biological and structural characterization of new linear gomesin analogues with improved therapeutic indices. Biopolymers

Henriques ST, Melo MN, Castanho MA (2006) Cell-penetrating peptides and antimicrobial peptides: how different are they? Biochem J 399:1–7

Hofmann MW, Weise K, Ollesch J, Agrawal P, Stalz H, Stelzer W, Hulsbergen F, de Groot H, Gerwert K, Reed J, Langosch D (2004) De novo design of conformationally flexible transmembrane peptides driving membrane fusion. Proc Natl Acad Sci USA 101:14776–14781

Hsu CH, Chen C, Jou ML, Lee AY, Lin YC, Yu YP, Huang WT, Wu SH (2005) Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: evidence for multiple conformations involved in binding to membranes and DNA. Nucleic Acids Res 33:4053–4064

Huang HW (2006) Molecular mechanism of antimicrobial peptides: the origin of cooperativity. Biochim Biophys Acta 1758:1292–1302

Jelinek R, Kolusheva S (2005) Membrane interactions of host-defense peptides studied in model systems. Curr Protein Pept Sci 6:103–114

Jin Y, Hammer J, Pate M, Zhang Y, Zhu F, Zmuda E, Blazyk J (2005) Antimicrobial activities and structures of two linear cationic peptide families with various amphipathic beta-sheet and alpha-helical potentials. Antimicrob Agents Chemother 49:4957–4964

Kim S, Jeon TJ, Oberai A, Yang D, Schmidt JJ, Bowie JU (2005) Transmembrane glycine zippers: physiological and pathological roles in membrane proteins. Proc Natl Acad Sci USA 102:14278–14283

Ladokhin AS, White SH (1999) Folding of amphipathic alpha-helices on membranes: energetics of helix formation by melittin. J Mol Biol 285:1363–1369

Leite JR, Silva LP, Rodrigues MI, Prates MV, Brand GD, Lacava BM, Azevedo RB, Bocca AL, Albuquerque S, Bloch C Jr (2005) Phylloseptins: a novel class of anti-bacterial and anti-protozoan peptides from the Phyllomedusa genus. Peptides 26:565–573

Lorizate M, de la Arada I, Huarte N, Sanchez-Martinez S, de la Torre BG, Andreu D, Arrondo JL, Nieva JL (2006) Structural analysis and assembly of the HIV-1 Gp41 amino-terminal fusion peptide and the pretransmembrane amphipathic-at-interface sequence. Biochemistry 45:14337–14346

Lotta TI, Salonen IS, Virtanen JA, Eklund KK, Kinnunen PK (1988) Fourier transform infrared study of fully hydrated dimyristoylphosphatidylglycerol. Effects of Na+ on the sn-1′ and sn-3′ headgroup stereoisomers. Biochemistry 27:8158–8169

Magzoub M, Eriksson LE, Graslund A (2002) Conformational states of the cell-penetrating peptide penetratin when interacting with phospholipid vesicles: effects of surface charge and peptide concentration. Biochim Biophys Acta 1563:53–63

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

Nagpal S, Kaur KJ, Jain D, Salunke DM (2002) Plasticity in structure and interactions is critical for the action of indolicidin, an antibacterial peptide of innate immune origin. Protein Sci 11:2158–2167

Nes IF, Holo H (2000) Class II antimicrobial peptides from lactic acid bacteria. Biopolymers 55:50–61

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

Noinville S, Bruston F, El Amri C, Baron D, Nicolas P (2003) Conformation, orientation, and adsorption kinetics of dermaseptin B2 onto synthetic supports at aqueous/solid interface. Biophys J 85:1196–1206

Oren Z, Lerman JC, Gudmundsson GH, Agerberth B, Shai Y (1999) 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(Pt 3):501–513

Papo N, Shai Y (2003) Can we predict biological activity of antimicrobial peptides from their interactions with model phospholipid membranes? Peptides 24:1693–1703

Pierre TN, Seon AA, Amiche M, Nicolas P (2000) Phylloxin, a novel peptide antibiotic of the dermaseptin family of antimicrobial/opioid peptide precursors. Eur J Biochem 267:370–378

Powers JP, Hancock RE (2003) The relationship between peptide structure and antibacterial activity. Peptides 24:1681–1691

Raguse TL, Porter EA, Weisblum B, Gellman SH (2002) Structure-activity studies of 14-helical antimicrobial beta-peptides: probing the relationship between conformational stability and antimicrobial potency. J Am Chem Soc 124:12774–12785

Ramirez-Alvarado M, Blanco FJ, Niemann H, Serrano L (1997) Role of beta-turn residues in beta-hairpin formation and stability in designed peptides. J Mol Biol 273:898–912

Rao AG (1999) Conformation and antimicrobial activity of linear derivatives of tachyplesin lacking disulfide bonds. Arch Biochem Biophys 361:127–134

Reichert J, Grasnick D, Afonin S, Buerck J, Wadhwani P, Ulrich AS (2007) A critical evaluation of the conformational requirements of fusogenic peptides in membranes. Eur Biophys J

Rinaldi AC (2002) Antimicrobial peptides from amphibian skin: an expanding scenario. Curr Opin Chem Biol 6:799–804

Robinson WE Jr, McDougall B, Tran D, Selsted ME (1998) Anti-HIV-1 activity of indolicidin, an antimicrobial peptide from neutrophils. J Leukoc Biol 63:94–100

Sato H, Feix JB (2006) Peptide-membrane interactions and mechanisms of membrane destruction by amphipathic alpha-helical antimicrobial peptides. Biochim Biophys Acta 1758:1245–1256

Shai Y (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochim Biophys Acta 1462:55–70

Stahelin RV, Cho W (2001) Differential roles of ionic, aliphatic, and aromatic residues in membrane-protein interactions: a surface plasmon resonance study on phospholipases A2. Biochemistry 40:4672–4678

van Kan EJ, van der Bent A, Demel RA, de Kruijff B (2001) Membrane activity of the peptide antibiotic clavanin and the importance of its glycine residues. Biochemistry 40:6398–6405

Vanhoye D, Bruston F, Nicolas P, Amiche M (2003) 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 270:2068–2081

Vanhoye D, Bruston F, El Amri S, Ladram A, Amiche M, Nicolas P (2004) Membrane association, electrostatic sequestration, and cytotoxicity of Gly-Leu-rich peptide orthologs with differing functions. Biochemistry 43:8391–8409

Wieprecht T, Dathe M, Beyermann M, Krause E, Maloy WL, MacDonald DL, Bienert M (1997) Peptide hydrophobicity controls the activity and selectivity of magainin 2 amide in interaction with membranes. Biochemistry 36:6124–6132

Wimmer R, Andersen KK, Vad B, Davidsen M, Molgaard S, Nesgaard LW, Kristensen HH, Otzen DE (2006) Versatile interactions of the antimicrobial peptide novispirin with detergents and lipids. Biochemistry 45:481–497

Zelezetsky I, Tossi A (2006) Alpha-helical antimicrobial peptides-Using a sequence template to guide structure-activity relationship studies. Biochim Biophys Acta 1758:1436–1449

Zelezetsky I, Pacor S, Pag U, Papo N, Shai Y, Sahl HG, Tossi A (2005) Controlled alteration of the shape and conformational stability of alpha-helical cell-lytic peptides: effect on mode of action and cell specificity. Biochem J 390:177–188