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Investigating the role of GXXXG motifs in helical folding and self-association of plasticins, Gly/Leu-rich antimicrobial peptides ☆

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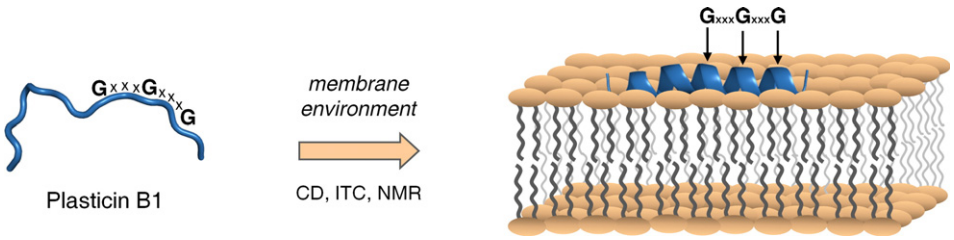
HIGHLIGHTS

- Plasticins have much higher affinity for anionic lipids than zwitterionic lipids.
- The two Gly-rich plasticins fold into well-defined helices in membrane environments.
- In micelles, Gly residues are located on the polar face of the amphipathic helix.
- GXXXG motifs in plasticins do not promote strong association between helices.
- The role of GXXXG motifs in plasticin could differ from that in transmembrane helices.

GRAPHICAL ABSTRACT



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ABSTRACT

Plasticins (PTC) are dermaseptin-related antimicrobial peptides characterized by a large number of leucine and glycine residues arranged in GXXXG motifs that are often described to promote helix association within biological membranes. We report the structure and interaction properties of two plasticins, PTC-B1 from *Phyllomedusa bicolor* and a cationic analog of PTC-DA1 from *Pachymedusa dacnicolor*, which exhibit membrane-lytic activities on a broad range of microorganisms. Despite a high number of glycine, CD and NMR spectroscopy show that the two plasticins adopt mainly alpha-helical conformations in a wide variety of environments such as trifluoroethanol, detergent micelles and lipid vesicles. In DPC and SDS, plasticins adopt well-defined helices that lie parallel to the micelle surface, all glycine residues being located on the solvent-exposed face. Spectroscopic data and cross-linking experiments indicate that the GXXXG repeats in these amphipathic helices do not provide a strong oligomerization interface, suggesting a different role from GXXXG motifs found in transmembrane helices.

Abbreviations: 1D, one dimensional; 2D, two dimensional; CD, circular dichroism; CSD, chemical shift deviation; DPC, dodecylphosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phospho-1′-*rac*-glycerol; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; HSQC, heteronuclear single quantum correlation spectroscopy; ITC, isothermal titration calorimetry; LUV, large unilamellar vesicle; MD, molecular dynamics; MLV, multilamellar vesicle; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; PBS, phosphate buffered saline; PFG, pulse field gradient; POPG, 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphoglycerol; PTC, plasticin; RMSD, root mean square deviation; SDS, sodium dodecyl sulfate; TFE, trifluoroethanol; TOCSY, total correlation spectroscopy.

☆ This paper is dedicated to the memory of Francine Bruston, who made significant contributions in the field of plasticins.

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1. Introduction

Membrane-active antimicrobial peptides are key elements of innate immunity in most animals, from insects to vertebrates [1,2]. The skin of amphibian, in particular, constitutes a rich source of peptides, with a large number and variety of secreted peptides that can be active on Gram-positive and Gram-negative bacteria, yeasts, fungi and protozoa [3,4]. The isolation and characterization of these antimicrobial peptides has aroused considerable interest with the emergence of numerous microbial strains resistant to conventional antibiotics [5–7].

Plasticins (PTC) are antimicrobial peptides found in the skin secretions of South-American hylid frogs [8,9]. Several members of this family have been identified in species belonging to the genera *Agalychnis*, *Leptodactylus*, *Pachymedusa*, and *Phyllomedusa* [10,11]. These peptides belong to the dermaseptin superfamily of host-defense peptides [12] that are characterized by a strong sequence conservation of signal peptide and precursor proregions but more divergent sequences of the mature peptides [13]. PTCs can be distinguished from other members of the dermaseptin superfamily by a large number of Gly and Leu residues in their sequences [14]. In addition, Gly residues are often arranged in regular 5-mer GXXXG motifs (where X is any amino acid residue). PTCs are typically 23–29 residues in length and contain 1 to 4 GXXXG motifs.

Peptides within PTC family differ markedly in their net charge owing to a different number of positively charged Lys residues, yielding divergent lytic activities. Cationic PTCs such as PTC-B1 isolated from *Phyllomedusa bicolor* and PTC-S1 from *P. sauvagii* exhibit a broad spectrum of antimicrobial activity at micromolar concentrations. In contrast, neutral or weakly cationic PTCs such as PTC-A1 from *Agalychnis annae*, PTC-C1 and PTC-C2 from *A. callidryas*, PTC-DA1 from *Pachymedusa dacnicolor*, and PTC-L1 from *Leptodactylus laticeps* are not potent antimicrobial peptides and tend to be moderately hemolytic. These neutral PTCs may be endowed with other biological properties such as immunomodulation, as demonstrated for PTC-L1 [15], and may act synergistically with other cationic peptides to lyse microorganisms.

The importance of cationic charges in the modulation of biological properties was further demonstrated by incorporating Lys residues in the sequence of neutral PTCs. In particular, we found that substituting three positions in PTC-DA1 by corresponding Lys and Phe residues in PTC-B1 ortholog (Table 1) yielded a cationic peptide [$K^{8,12}$,F^{18}]PTC-DA1 with enhanced antimicrobial potency [10]. The properties of PTC-B1 and the synthetic analog [$K^{8,12}$,F^{18}]PTC-DA1 were extensively studied in order to analyze their interactions with lipids at membrane interfaces and decipher their membrane-disruptive mode of action in relation with their antimicrobial potencies [16–18]. These peptides exhibit differential membrane-lytic activities on a wide range of microorganisms including methicillin-resistant *Staphylococcus aureus* strains [17].

The mode of action of most dermaseptin-related antimicrobial peptides from hylid frogs is believed to be the permeation or disruption of the lipid plasma membrane of the target cells. Several mechanisms have been proposed such as the barrel stave or toroidal pore models, the carpet or detergent models, and the lipid clustering model [19,20]. The involved mechanisms are dependent both on lipid composition and peptide properties with respect to secondary structure, conformational flexibility, charge, hydrophobicity, amphipathicity, and peptide aggregation. Numerous antimicrobial peptides have been reported to adopt amphipathic α-helical structures in membrane environments. Although helical structures have been evidenced for PTC-B1 and [$K^{8,12}$,F^{18}]PTC-DA1 in lipid environments, the conformations adopted by members of the PTC family are markedly dependent on the environment [8]. Indeed PTCs can display random coil, α-helical, β-sheet or β-hairpin structures, revealing their huge conformational plasticity. The large number of Gly residues, which have inherent flexibility, could be an important element of this structural versatility.

Another intriguing property of PTCs is the presence of repeated GXXXG motifs. To this respect, the amino acid sequences of PTCs resemble those of transmembrane protein segments, in which GXXXG motifs are known to mediate interactions between transmembrane helices and promote helix bundle formation [21–23]. GXXXG motifs are prevalent in membrane proteins but they are also observed in soluble proteins in which they can participate in helix-helix interactions [24]. Since peptide self-association is a prerequisite in several proposed mechanisms of membrane permeation, these GXXXG motifs could be involved in peptide oligomerization. Indeed, it has previously been proposed that the central GXXXG motif of PGLa antimicrobial peptide is involved in the formation of an antiparallel helix dimer in the membrane-embedded tilted T-state [25,26]. Other antimicrobial peptides containing GXXXG or AXXXA motifs such as bacteriocins [27] or bombinins [28] also exhibit oligomerization properties. Nevertheless, in the case of PTCs, the involvement of GXXXG motifs in helical bundle formation has not been investigated so far.

The aim of the present work was to analyze the interactions and the conformations of two selected PTCs, PTC-B1 and [$K^{8,12}$,F^{18}]PTC-DA1 analog, in membrane-mimetic environments using ITC, CD and NMR spectroscopy. In particular, we examined to which extent the high number of Gly residues could influence helical folding of PTCs. We also investigated whether the presence of three repeated GXXXG motifs in the two PTCs sequences (Table 1) could promote their self-association in membrane environments.

2. Experimental

2.1. Solid phase peptide synthesis

Peptides (0.1 mmol) were synthesized using FastMoc chemistry on an Applied Biosystems 433A automated peptide synthesizer (Applera, France) as described [10]. Briefly, PTC-B1 was prepared on a Fmoc-Ser(*t*Bu)-Novasyn TGA resin substituted at 0.22 mmol/g (Novabiochem-Merck, Germany). [$K^{8,12}$,F^{18}]PTC-DA1 was synthesized using Rink amide MBHA PS resin substituted at 0.85 mmol/g (Senn Chemicals). Amino acids were purchased from Novabiochem. Peptides were purified by reverse-phase high performance liquid chromatography (RP-HPLC) on a semi-preparative C18 column and their identity and purity were assessed by MALDI-TOF mass spectrometry. Synthesis and purification were performed by the Platform "Synthèse peptidique" of IBPS/FR 3631 Institut de Biologie Paris-Seine (Université Pierre et Marie Curie, Paris).

Table 1
Amino acid sequence and physicochemical characteristics of plasticins investigated in this study.

Peptide[a]	Sequence[b]	Net charge[c]	Mean hydrophobicity[d]	Mean hydrophobic moment (μH)[d]
PTC-B1	GLVTSLIKGA**G**KLL**G**GLF**G**SVT**G**GQS	+2	0.33	3.0
[$K^{8,12}$,F^{18}]PTC-DA1	GVVTDLLKTA**G**KLL**G**NLF**G**SLS**G**-NH_2	+2	0.5	3.96

[a] Plasticins have been renamed according to the new nomenclature proposed by Amiche et al. [9]. PTC-B1 and PTC-DA1 correspond to the formerly named DRP-PBN2 and DRP-PD3-6 peptides, respectively. The analog [$K^{8,12}$,F^{18}]PTC-DA1 was previously called DRP-PD36KF.
[b] Glycines involved in **G**XXX**G** motifs are indicated in bold.
[c] The net charge of the plasticins is given for pH 7.
[d] Mean hydrophobicities and hydrophobic moments (using the CSS scale) were calculated using HydroMCalc program (http://www.bbcm.univ.trieste.it/~tossi/HydroCalc/HydroMCalc.html).