Volume 228, number 2, 337-340 FEB 05561 February 1988

Antimicrobial properties of peptides from *Xenopus* granular gland secretions

Emilia Soravia, Giuseppe Martini and Michael Zasloff

Human Genetics Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

Received 15 December 1987

Previously, we described a family of novel broad spectrum antimicrobial peptides, magainins, from the skin of *Xenopus laevis*. In this report we show that at least two other *Xenopus* peptides, present in the skin and its secretions, PGLa and a peptide released from the xenopsin precursor, exhibit antimicrobial properties comparable to the magainins. The identification of these newer members provides insight into the structural diversity of vertebrate antimicrobial peptides.

Antimicrobial peptide; Magainin; Antibiotic; (*Xenopus*)

1. INTRODUCTION

We have previously reported the isolation of two abundant antimicrobial peptides called 'magainins' from the skin of the South African clawed toad, *Xenopus laevis* [1,2]. Recently, a comprehensive analysis of peptides present in the secretions of the granular glands of the skin of *Xenopus* was published [3]. Major components of these secretions were shown to include the magainin peptides (called 'PGS' in [3]), the 21 residue peptide PGLa [3–6], and a 25 residue peptide which derives from the xenopsin precursor (XPF) [3,6,7]. PGLa and XPF have no known function but have been recognized as potentially membrane-active substances [3–6]. Although these peptides share no significant sequence similarity to the magainins, their similar size, potential amphilicity, abundance, and glandular location suggested to us that they might share with the magainins a common antimicrobial activity. In this report we show that both PGLa and XPF exhibit broad spectrum antimicrobial activity comparable to the magainins. The results demonstrate that the magainins represent members of a surprisingly diverse population of antimicrobial peptides present within the amphibian neurosecretory structure.

Correspondence address: E. Soravia, Human Genetics Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

2. MATERIALS AND METHODS

Antibacterial and antiprotozoan activity were assayed as described [1,2]. Peptides were synthesized by the solid-phase procedure [8] as described [2,5] by Dr Jean River (Salk Institute, La Jolla, CA) and Michael Brasseur (Applied Biosystems, Foster City, CA).

3. RESULTS

PGLa, the xenopsin precursor fragment (XPF) and magainin 2 were synthesized as the carboxyl-terminal amides by the solid-phase method [8] as described [2,5] and purified to homogeneity by reverse-phase high-pressure liquid chromatography [2,5]. Sequences of these peptides are shown in table 1.

The synthetic peptides were assayed for antimicrobial activity by methods described in [1,2]. When applied to a lawn of *E. coli*, XPF, PGLa and magainin 2 displayed comparable antibacterial

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

Table 1

Sequences of the natural peptides

Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS [1-3]
XPF	GWASKIGQTLGKIAKVGLKELIQPK [3]
PGLa	GMASKAGAIAGKIAKVALKAL (NH_2) [4]

The synthetic peptides used in this report all bear carboxyl-terminal amides

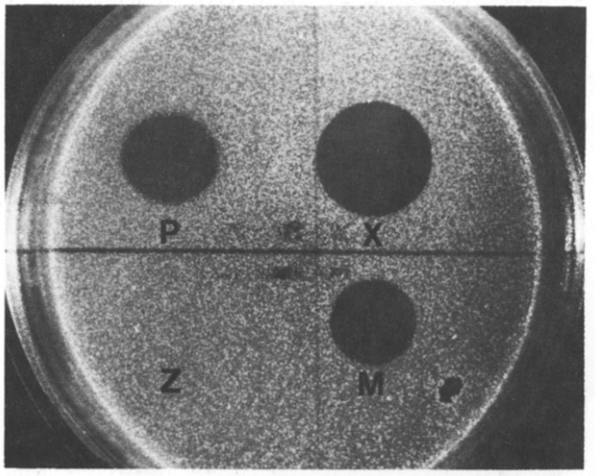

Fig.1

activities (fig.1). An analogue of magainin 2, in which all Lys and Phe residues had been synthesized with D enantiomers (peptides Z), displayed markedly reduced activity, serving as control and demonstrating the specificity of the antimicrobial assay.

When assayed against several species of bacteria and fungi in liquid medium, both XPF and PGLa exhibited antimicrobial potencies comparable to that observed for magainin 2 (table 2). However, the precise spectrum of antibiotic activity differed between the peptides. Thus, magainin 2 and XPF displayed higher specific activity against *Pseudomonas* than did PGLa, while PGLa was the most active against *Staphylococcus aureus*. At minimal inhibitory concentrations, all three peptides exhibited bactericidal activity against the organisms assayed in table 2, with greater than 90% killing observed by the first hour of incubation (not shown).

Synthetic magainin peptides were previously shown to rapidly induce osmotic lysis of several protozoan species [2]. Consequently, both XPF and PGLa were assayed for similar antiprotozoan activity. As shown in table 3, XPF and PGLa exhibited potent activity against *Paramecium caudatum, Acanthamoeba castellani* and *Tetrahymena pyriformis*. As seen with magainin peptides, both PGLa and XPF rapidly inhibited contractile vacuole function, resulting in progressive swelling of the organism followed by eventual rupture. It would appear that each of the three peptides acts on a common intracellular site in this complex microbial target.

The amino acid sequences of magainin, PGLa and XPF permit them to be configured into an am-

Table 2

Spectrum of antimicrobial activity of *Xenopus* granular gland peptides

Organism (ATCC)	Minimal inhibitory concentration (μg/ml)			
	Magainin 2	PGLa	XPF	Z
Escherichia coli (25922)	10– 50	10– 50	10– 50	>1000
Pseudomonas aeruginosa (27883)	50–100	200–500	50–100	>1000
Staphylococcus aureus (25923)	>500	50–100	100–200	>1000
Streptococcus pyogenes (19615)	10– 50	10– 50	10– 50	>1000
Saccharomyces cerevisiae (A3180)	50–100	100–200	200–500	>1000
Candida albicans (14053)	200–500	100–200	200–500	>1000

10^5 organisms were inoculated from late-log phase cultures into 0.5 ml of TSB broth containing synthetic peptides at concentrations of 10, 50, 100, 200, 500 and 1000 μg/ml. Cultures were incubated at either 37°C (bacteria) or 30°C (fungi) for 24 h. Concentration range of peptide at which no visible growth of inoculum occurred is listed. Peptide Z is [D-Lys^{4,10,11,14},D-Phe^{5,12,16}]-magainin 2 carboxy-terminal amide

Table 3

Sensitivity of protozoa to synthetic *Xenopus* granular gland peptides

Organism	Minimal disruptive concentration (μg/ml)			
	Magainin 2	PGLa	XPF	Z
Paramecium caudatum	10	5	10	>250
Tetrahymena pyriformis	20	20	20	>250
Acanthamoeba castellani	2	2	2	30

Protozoa (about 10^2) were suspended into 200 μl of 1% trypticase soy broth in distilled water on a glass depression slide. Actively growing cultures of *P. caudatum* and *T. pyriformis* were obtained from Nasco (WI) and *A. castellani* from B. Bowers (NHLBI). Peptides were added to various concentrations and the organisms visually assessed by light microscopy within 15 min of exposure. The concentrations listed represent the minimal peptide concentration at which physical disruption of all protozoa occurred

phiphilic α-helix [1–6]. Indeed, we have recently demonstrated by two dimensional nuclear magnetic resonance spectroscopy, that magainin 2 adopts an α-helical structure in the presence of low concentrations of trifluoroethanol [12]. Although amphiphilic peptides can be membrane disruptive, the magainins have been shown previously to exhibit little hemolytic activity against human erythrocytes [1]. As seen in table 4, both PGLa and XPF, exhibit very low hemolytic activity, quantitatively identical to magainin 2. As a class, therefore, they are distinguished from lytic peptides, such as mellitin [9], and bombinin [10], which are potent cytotoxic hemolytic substances.

Table 4

Hemolytic activity of *Xenopus* peptides

Peptide (μg/ml)	Peptide (% hemolysis)			
	Magainin 2	PGLa	XPF	Mellitin
0	0	0	0	0
50	4	4	1	100
100	3	3	1	–
200	3	4	2	–
500	4	5	5	–
1000	6	7	6	–

Peptides, at the stated concentrations, were added to a 5% suspension of freshly drawn human erythrocytes, which had been washed twice in phosphate buffered saline. After incubation at 37°C for 30 min, the suspension was centrifuged at 10000 × *g* for 10 min and the A_{400} of the supernatant determined. 100% hemolysis was determined by addition of 0.2% Triton X-100 to a standard reaction

4. DISCUSSION

In this report we have shown that in addition to the magainins, at least two other peptides found in the secretion of granular gland of *Xenopus laevis* exhibit broad-spectrum antimicrobial activity. Our failure to detect these species during our initial analysis of antimicrobial activity in *Xenopus* skin extracts probably reflects the relatively greater abundance of the magainin peptides [3] and selective losses of PGLa and XPF during extraction and purification. Although we have examined only two granular gland peptides for antimicrobial activity, other candidates with potential comparable activities may well be present in the skin. It appears that antimicrobial peptides may represent a larger family than initially expected in this amphibian. The basis for this diversity remains unclear. If, however, these peptides serve an antimicrobial function in vivo, together they may provide a more comprehensive spectrum of action than afforded by an individual peptide.

Despite the common antimicrobial property exhibited by these peptides it is striking that as a group they do not share a common amino acid sequence of any significant length (table 1). The extensive homology observed between XPF and PGLa has been noted previously and extends over much of the length of the corresponding precursors [7]. However, as a group, these peptides clearly share certain features that must play a role in their antimicrobial activity: they are at least 21 residues in length, sufficient to span a lipid bilayer in an α-helical configuration [10]; they can be configured into α-helical structures in which hydrophilic side chains align along one face, while hydrophobic side chains align along the other, a property of membrane-active peptides [10]; they are composed of predominantly basic and hydrophobic residues. Recent conductance studies utilizing magainin 2 have demonstrated the capacity of this peptide to form voltage-dependent anion-specific channels in synthetic bilayers (Cruciani et al., in preparation). To explain this property, which may underlie the antimicrobial activity of magainin, we have speculated that individual

monomers, configured as α-helical structures, organize to form a transmembrane channel (Feldmann, R. et al., unpublished). Whether PGLa and XPF, which can be modelled into similar channel structures, exhibit similar ionophoric properties is currently under study.

The localization of an antimicrobial peptide, XPF, as part of the same precursor as xenopsin, an 8 residue vasoactive peptide [11] is intriguing. Xenopsin has been identified in many tissues of *Xenopus* in addition to skin, including gut and brain [11]. Its widespread distribution and biological activity has led to the suggestion that xenopsin may play a role in wound repair and inflammation, as a regulator of local blood flow and vascular permeability [11]. The demonstration that the xenopsin precursor yields an antimicrobial peptide in addition to xenopsin, further supports a role for this family of peptides in wound repair and defense.

Acknowledgements: We wish to acknowledge Dr John Spencer and Dr Charles Bevins for critical reading of the manuscript and Ms Alina Patton for help in typing the manuscript.

REFERENCES

[1] Zasloff, M. (1987) Proc. Natl. Acad. Sci. USA 84, 5449–5453.
[2] Zasloff, M., Martin, B. and Chen, H.C. (1987) Proc. Natl. Acad. Sci. USA, in press.
[3] Giovannini, M.G., Poulter, L., Gibson, B.W. and Williams, D.H. (1987) Biochem. J. 243, 113–120.
[4] Hoffman, W., Richter, K. and Kreil, G. (1983) EMBO J. 2, 711–714.
[5] Andreu, B., Aschauer, H., Kreil, G. and Merrifield, R.B. (1985) Eur. J. Biochem. 149, 531–535.
[6] Gibson, B.W., Poulter, L., Williams, D.H. and Maggio, J.E. (1986) J. Biol. Chem. 261, 5341–5349.
[7] Sures, I. and Crippa, M. (1984) Proc. Natl. Acad. Sci. USA 81, 380–384.
[8] Merrifield, R.B. (1963) J. Am. Chem. Soc. 85, 2149–2156.
[9] Eisenberg, D. (1984) Annu. Rev. Biochem. 53, 595–623.
[10] Csordas, A. and Michl, H. (1970) Montash. Chem. 101, 182–189.
[11] Carraway, R.E., Cochrane, D.E. and Ruane, S.E. (1987) J. Biol. Chem. 262, 15886–15889.
[12] Marion, D., Zasloff, M. and Bax, A. (1987) FEBS Lett. 227, 21–26.