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Host-defense peptides of the skin with therapeutic potential: From hagfish to human

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ABSTRACT

It is now well established that peptides that were first identified on the basis of their ability to inhibit growth of bacteria and fungi are multifunctional and so are more informatively described as host-defense peptides. In some cases, their role in protecting the organism against pathogenic microorganisms, although of importance, may be secondary. A previous article in the journal (Peptides 2014; 57:67–77) assessed the potential of peptides present in the skin secretions of frogs for development into anti-cancer, antiviral, immunomodulatory and antidiabetic drugs. This review aims to extend the scope of this earlier article by focusing upon therapeutic applications of host-defense peptides present in skin secretions and/or skin extracts of species belonging to other vertebrate classes (Agnatha, Elasmobranchii, Teleostei, Reptilia, and Mammalia as represented by the human) that supplement their potential role as anti-infectives for use against multidrug-resistant microorganisms.

Introduction

Q2 Cell-penetrating peptides with varying degrees of cytotoxicity play an important role in the system of innate immunity that predates adaptive immunity and constitutes the first-line defense against invading pathogens for all classes of vertebrate species. As might be expected, these peptides are synthesized primarily in tissues that interface directly with the environment so that skin (as well as intestine and lung/gill) represents a major site of production and they are often found in high concentrations in sweat and skin secretions. Such peptides were first identified on the basis of their abilities to inhibit the growth of bacteria and/or fungi and so are generally referred to as "antimicrobial peptides". However, it is now well established that these components are multi-functional [21,29,66,129]. Many show potent cytotoxicity against human-derived tumor cells and viruses as well as displaying cytokine-mediated immunomodulatory activities, chemokine-mediated chemoattractive properties, and both mast cell degranulating and insulin-releasing activities. The more toxic peptides may also play a role in deterring predators [65]. Consequently, it is more informative to refer to them as "host-defense peptides".

A comparison of amino acid sequences reveals that evolutionary pressure to conserve the primary structures of the host-defense peptides of vertebrates has been extremely weak. It is not possible to identify invariant or strongly conserved domains that are associated with biological activity. This observation is consistent with the mechanisms by which the peptides produce their biological effects which most commonly involve a non-specific interaction with the bacterial cell membrane or with intracellular targets rather than binding to a specific receptor [4]. However, the host-defense peptides produced in the skin are associated with certain common physicochemical features. The vast majority of such peptides are cationic. Of the antimicrobial peptides listed in the Antimicrobial Peptide Database [112], approximately 90% are cationic with an average net charge of +4 at pH 7. The peptides that act by disruption of the integrity of the cell membrane generally contain more than 40% hydrophobic amino acids, but effects of hydrophobicity on antimicrobial activity are less pronounced as electrostatic interactions of the peptide with the negatively charged bacterial cell membrane predominate [18].

Although the β-strand structural motif is found in several vertebrate antimicrobial peptides, such as the defensins, the conformational feature that predominates among the peptides found in the skin is the amphipathic α-helix in which there is segregation of the polar residues on one face of the helix and the hydrophobic residues on the opposite face. The peptides generally exist in a random coil conformation in aqueous solution but adopt the helical conformation in a membrane-mimetic solvent, such as 50% trifluoroethanol–water, or on binding to the bacterial cell membrane. However, interaction is rarely specific for the prokaryotic membrane and host-defense peptides show varying degrees of

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cytotoxicity for eukaryotic cells. Structure–activity studies show that both helicity and amphipathicity correlate directly with the ability to lyse mammalian cells. Small increases in the hydrophobic moment (a semi-quantitative measure of the amphipathicity of a helical peptide) may produce major changes in activity (reviewed in [18]). Because they are readily incorporated into a helix, leucine, alanine, and lysine frequently occur in skin antimicrobial peptides but many contain a proline residue, necessary for high potency, that produces a kink or bend in the molecule that facilitates membrane penetration [103].

Histones and peptides derived from histones by proteolytic cleavage constitute a second category of host-defense peptides found in epidermal secretions. Post-translationally modified histones are a major component of eukaryotic chromatin but unmodified or minimally modified histones are also present in the cytoplasm [63]. These components display varying degrees of growth inhibitory activity against bacteria and "extranucleosomal" histones play important roles in host defense, particularly in skins and epidermal mucus of teleost fish. Their mechanism of action has not been completely elucidated but may involve ATP-dependent translocation across the bacterial cell membrane without producing lysis and inhibition of cellular function by binding to intracellular DNA and/or RNA targets [64].

Without doubt, the host-defense peptides associated with the skins of Anura (frogs and toads) have received the most attention and more than 1000 such peptides with defined antimicrobial activity from this source are listed in the antimicrobial peptide database [112]. A number of recent articles have reviewed the species distribution, primary structures, and antimicrobial activities of these compounds [10,26,28,65]. Despite the considerable enthusiasm that accompanied the discovery of the magainins in skin secretion of *Xenopus laevis* in 1987 [47,132], it is fair to write that the promise of naturally occurring amphibian host-defense as effective anti-infective agents, even for topical applications, has not been fulfilled. The potencies and antimicrobial specificities of the frog skin peptides do not appear to be superior to those of short, rationally designed, synthetic amphipathic α-helical peptides [34]. Rather disturbingly, exposure of *Staphylococcus aureus* to the potent magainin analog, pexiganan resulted in development of resistance not only to this agent but also to human neutrophil defensin-1 suggesting that antimicrobial peptide therapy for infection may compromise natural immunity [51]. No agent derived from a frog skin peptide is currently being used in clinical practice. Consequently, attention is being increasingly directed toward other potential therapeutic applications.

The emergence of multidrug-resistant cancers and the lack of targeted therapies for many cancers necessitate a search for new therapeutics that are active against tumor cells. Similarly, the current pandemic of Type 2 diabetes mellitus has stimulated a search for natural products that increase insulin secretion and sensitivity. A recent article has assessed the possibility of developing host-defense peptides identified in frog skins into anticancer, antiviral, immunomodulatory, and antidiabetic agents [29]. This review aims to extend the scope of this earlier article by focusing upon possible therapeutic applications of host-defense peptides present in skin secretions and/or skin extracts of species belonging to other vertebrate classes: Agnatha (hagfish and lampreys), Elasmobranchii (sharks, rays, and skates), Teleostei (bony fish), Reptilia (snakes, lizards, and turtles), and Mammalia as represented by the human. The primary structures of the non-tetrapod skin peptides mentioned in the article are shown in Table 1 and the peptides from reptilian species and the human are shown in Table 2.

Agnatha

The Agnatha, or jawless fishes, represent the first vertebrate stock whose line of evolution diverged from that leading to gnathostomes at least 550 million years ago. The fossil record indicates that agnathans were numerous during the late Silurian/early Devonian (390–420 million years ago) but only the hagfishes (Myxinoformes) and lampreys (Petromyzontiformes) have survived until the present day. The system of adaptive immunity that characterizes the higher vertebrates is not fully developed in the Agnatha so that host-defense peptides of the innate immune system may be of particular importance in protecting the organism against infection [13]. Instead of the immunoglobulin based antigen receptors of gnathostomes, the Agnatha use variable lymphocyte receptors (VLRs) comprised of leucine-rich-repeat (LRR) segments for antigen recognition [121].

The taxonomy and phylogeny of the Agnatha is in a state of flux with several unresolved issues [43]. A total of 78 species of hagfish are currently recognized as valid and are distributed in up to seven genera in two subfamilies: Myxininae and Eptatretinae. In response to osmotic stress or attack by a predator, hagfish skin produces large volumes of viscous slime composed primarily of bulk seawater entrained between mucin-coated fine fibers known as slime threads [45]. While the primary biological function of the slime is probably to defend the hagfish by clogging the gills of predatory fish [135], it also contains components such as lysozyme and peptides derived from hemoglobin and histone 2B that may be involved in

Table 1
Host-defense peptides identified in the skins of non-tetrapod vertebrates and selected analogs.

Peptide	Species	Primary structure
Agnatha		
Myxinidin	*Myxine glutinosa*	GLHDILKYGKPS
LCRP	*Petromyzon marinus*	CPCGRRRCCVRGLNVYCCF
LCRP	*Lampetra fluviatilis*	CPCGRKRCCVRGLNVYCCA
Elasmobranchii		
Kenojeinin I	*Raja kenojei*	GKQYFPKVGGRLSGAPLAAKTHRRLKP.NH_2
Teleostei		
Piscidin 1	*Morone chrysops* × *M. saxatilis*	FFHHIFRGIVHVGKTIHRLVTG
Piscidin 4	*Morone chrysops* x *M. saxatilis*	FFRHLFRGAKAIFRGARQGXRAHKVVSRYRNRDV PETDNNQEEP
Epinecidin-1	*Epinephelus coioides*	GFIFHIIKGLFHAGKMIHGLV.NH_2
Chrysophsin-1	*Chrysophrys major*	FFGWLIKGAIHAGKAIHGLIHRRRH.NH_2
Pardaxin	*Pardachirus marmoratus*	GFFALIPKIISSPLFKTLLSAVGSALSSSGDQE
Pleurocidin	*Pleuronectes americanus*	GWGSFFKKAAHVGKHVGKAALTHYL
Pleurocidin NRC-03	Synthetic analog	GRRKRKWLRRIGKGVKIIGGAALDHL.NH2
Pleurocidin NRC-04	Synthetic analog	GWGSFFKKAAHVGKHVGKAALTHYL.NH_2
Pleurocidin NRC-07	Synthetic analog	RWGKWFKKATHVGKHVGKAALTAYL.NH_2

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