Proline-Rich Antimicrobial Peptides: Multiple Potential Therapeutics Against Antibiotic Resistant Bacteria

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Abstract

The increasing resistance of pathogens to antibiotics is causing a huge clinical burden that is placing great demands on academic researchers and the pharmaceutical industry for resolution. Antimicrobial peptides, part of native host defense, have emerged as novel potential antibiotic alternatives. Among the different classes of antimicrobial peptides, proline-rich antimicrobial peptides, predominantly sourced from insects, have been extensively investigated to study their specific modes of action. In this review, we focus on recent developments in these peptides. They show a variety of modes of actions, including mechanism shift at high concentration, non-lytic mechanisms, as well as possess different intracellular targets and lipopolysaccharide binding activity. Furthermore, proline-rich antimicrobial peptides display the ability to not only modulate the immune system *via* cytokine activity or angiogenesis but also possess properties of penetrating cell membranes and crossing the blood brain barrier suggesting a role as potential novel carriers. Ongoing studies of these peptides will likely lead to the development of more potent antimicrobial peptides that may serve as important additions to the armoury of agents against bacterial infection and drug delivery.

Keywords: proline-rich antimicrobial peptides, non-lytic mechanism, membrane disruption, immunostimulation, cell penetration

Abbre*via*tions: AMPs, antimicrobial peptides; PrAMPs, proline-rich antimicrobial peptides; LPS, lipopolysaccharide; CPPs, cell penetrating peptides; BBB, blood brain barrier.

The trend of antimicrobial resistance development to conventional antibiotics has increased more rapidly, despite the availability of new classes antibiotics launched in recent years (Butler et al. 2013). The period of adapting resistance to these antibiotics is also becoming increasingly short (Hede 2014; Hsueh 2012; Zhang et al. 2006) (Figure 1). The resistance rates of *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* have accelerated beyond country borders *via* human activity and avian migration (Kanthor 2014). The widespread feeding of livestock with various antibiotics to aid their growth has also contributed to the enhancement of antibiotic resistance genes (Cully 2014; Zhu et al. 2013). Consequently, much research is ongoing to investigate the mechanism of antibiotic resistance and seeks to develop new antibiotics possessing various mechanisms of action (Cannon 2014; Gammon 2014).

Antimicrobial peptides (AMPs) are distributed in all living organisms as part of the host innate immunity (de Souza Cândido et al. 2014; Zasloff 2002). They have rapidly emerged as an important alternative to conventional antibiotics due to their potency and differing modes of action (Fox 2013; Hancock et al. 2012; Peters et al. 2010) which lead to broad spectrum activity against various microorganism (Brogden 2005; Lijuan Zhang 2006). Generally, AMPs have a net positive charge due to the presence of several lysines/arginines, and display amphipathic properties, which are crucial for the interaction with the microbial membrane and membrane binding (Fernández-Vidal et al. 2007; Hancock 1997; Powers and Hancock 2003). However, the composition and structure of AMPs vary considerably leading to different mechanisms of action. There are a number of reviews detailing their features, including toxicity, secondary structure and biological activity (Chen and Luo 2009; Giuliani et al. 2007; Jenssen et al. 2006; Splith and Neundorf 2011).

It has been postulated that membrane-active AMPs selectively disrupt the cell membrane to form pores that allow efflux of essential ions or nutrients (Matsuzaki 1999; Zasloff 2002). Based on the Shai-Matsuzaki-Huang (SMH) model, most AMPs act *via* an interaction with the membrane resulting in a morphological change of membrane structure (Matsuzaki 1999; Shai 1999; Yang et al.

2000). Different models of pore formation have been proposed and described in several reviews (Brogden 2005; Jenssen et al. 2006; Reddy et al. 2004; Shai 1999) (

Table **1**), and include barrel-stave (Matsuzaki et al. 1998), carpet (Dagan et al. 2002), toroidal pore or wormhole (Matsuzaki et al. 1996) and aggregate mechanism models (Wu et al. 1999) (Figure 2). However, there is increasing evidence that AMPs can kill targeted cells without causing membrane damage which suggests other potential intracellular targets and different mechanisms of killing (Bahar and Ren 2013; Brogden 2005; Otvos 2005) (

Table **1**). The intracellular targets can be divided into several groups, including inhibition of macromolecular (nucleic acid or protein) synthesis (Subbalakshmi and Sitaram 1998), inhibition of metabolic/enzymatic function (Otvos et al. 2000), and inhibition of cell-wall/membrane formation (Brötz et al. 1998) (Figure 1).

Many AMPs have been isolated from invertebrates, insects, plants and fungi, which are divided into groups, cationic AMPs and non-cationic AMPs with diversity of structure motifs including linear peptide of α-helices, β-strand stabilized via one or more disulfide bonds (Dimarcq et al. 1998; Pushpanathan et al. 2013). Based on their structural motif features, these AMPs display potent antimicrobial activity and specificity to the targeted cells. Beyond them, there are also some AMPs with high content of one or two amino acids, such as proline (Otvos 2002), arginine (Chan et al. 2006; Dong et al. 2012) and glycine (Baumann et al. 2010; Ilić et al. 2013). Among these different types of AMPs, proline-rich AMPs (PrAMPs) have attracted special attention due to their wide distribution in insects and unique mechanism of killing bacteria without cell membrane disruption (Otvos 2000). There are several reviews relating to the characteristics of PrAMPs, including isolation (Otvos 2000), non-lytic mechanism (Scocchi et al. 2011), intracellular targets (Otvos 2005; Otvos et al. 2000) and synergistic function (Cassone and Otvos 2010).

Mechanism of action

Previously, PrAMPs have been isolated from various species, including vertebrates (Agerberth et al. 1991; Gennaro et al. 1989; Gennaro et al. 2002; Shamova et al. 1999), crustaceans (Destoumieux et al. 2000; Stensvag et al. 2008), *anellidae* (Cho et al. 1998) and especially insects (Bulet et al. 1993; Bulet et al. 1999; Casteels et al. 1989; Otvos 2000). While the non-lytic mechanism of PrAMPs had been investigated extensively (Gennaro et al. 2002; Otvos 2002; Scocchi et al. 2011), additional PrAMPs possessing different actions have been both isolated and chemically synthesized. PP30, isolated from *Apocrita* and similar to abaecin, acts against microbes causing morphological changes and membrane damage (Shen et al. 2010). The designed PrAMP,

A3-APO and arasin 1, exhibit a dual mode of action with both membrane disruption and probable intracellular target inhibition (Paulsen et al. 2013; Rozgonyi et al. 2009). More interestingly, membrane destruction is concentration-dependent indicating that membrane permeabilization shifts to a membrane disruptive effect at high concentration (Hernandez-Gordillo et al. 2014; Paulsen et al. 2013; Podda et al. 2006; Végh et al. 2011). These findings will aid the development of more potent PrAMPs bearing a range of selective mechanisms of action.

Several reviews have discussed the action of intracellular inhibition in which PrAMPs show inhibition of the 70 kDa bacterial heat shock protein, DnaK, resulting in protein misfolding and aggregation and subsequent bacterial death (Otvos 2005). Furthermore, the binding site of inhibition was revealed *via* crystal structures of *Escherichia coli* Hsp70 chaperone DnaK (Otvos et al. 2000; Rozgonyi et al. 2009; Zahn et al. 2013; Zahn et al. 2014). However, there were additional indications of novel modes of action against the targeted bacteria. PrAMPs generally penetrate the outer membrane of Gram-negative bacteria and translocate *via* a permease/transporter-mediated uptake, but there is no increase in activity following engineered increased of the AMP cell penetration (Berthold and Hoffmann 2014). Furthermore, apidaecin IB was found not only to depress the GroEL/GroES chaperone team, but also resulted in the unbalanced biosynthesis of *Escherichia coli* lipopolysaccharide (LPS) and phospholipids (Zhou and Chen 2011). Moreover, Ls-Stylicin 1, which has a proline-rich N-terminal region, displayed potent LPS-binding activity *in vitro*, suggesting potential selective action against Gram-negative bacteria *via* cell wall interaction (Avitabile et al. 2014; Rolland et al. 2010). These findings indicate additional intracellular targets, beyond DnaK binding and inhibition, and novel modes of action including macromolecular biosynthesis inhibition and LPS-binding activity to attack targeted cells.

Immunostimulation

Native host defence peptides, produced in innate immune cells, display antimicrobial activity against bacteria and fungi associated with anti-inflammatory/immunomodulatory activity (Hancock

et al. 2012). These peptides have multiple mechanisms of action and possible additional therapeutic applications against antibiotic resistant bacteria (Hilchie et al. 2013; Wilson et al. 2013). Several PrAMPs have been shown to modulate systemic immunostimulation (Table 2). For instance, the proline-rich domain of penaeidin acts as a pro-inflammatory cytokine and modulated the immune system of the wound-induced inflammation response *via* autocrine activity (Li and Song 2010). Another PrAMP, Bac 7 (1-35), was shown to reduce the mortality of a pathogen infected mice model and modulated the defence response of macrophages after phagocytosis. As well, the cathelicidin-derived OaBac5mini showed the ability to suppress the inflammatory responses induced by pathogens (Pelillo et al. 2014; Yu et al. 2010). Furthermore, the designed PrAMP A3-APO showed more efficient *in vivo* than *in vitro* bactericidal abilities, which had been demonstrated in an infection model mouse with immunostimulatory properties *via* deactivating bacterial toxins, as well as upregulating the expression of anti-inflammatory cytokines, interleukin-4 and interleukin-10 (Ostorhazi et al. 2011; Otvos et al. 2014; Szabo et al. 2010). On the other hand, PR 39 not only acted as the inhibitor proteasome, but as the inducer of syndecans and angiogenesis to assist cell proliferation or new blood vessel growth (Anbanandam et al. 2008; Carmeliet 2000; Gaczynska et al. 2003; Gallo et al. 1994; Gennaro et al. 2002). PrAMPs have also been shown to modulate the expression of inflammatory cytokines and upregulate the wound repair process, which attack pathogens synergistically, suggesting a novel strategy to design and develop potent PrAMPs with enhanced immunomodulatory activity.

Drug delivery properties of PrAMPs

A common challenge for drug development is its distribution for treatment of disease. Cell penetrating peptides (CPPs) have shown promise as a means to deliver drugs to both bacterial and eukaryotic cells (Stewart et al. 2008), as well as for crossing the blood brain barrier (BBB) (Lalatsa et al. 2014). As discussed above, the mechanism of PrAMPs may not only involve membrane damage but also membrane penetration into cells. This indicates that much peptides have significant

potential as CPPs or transport systems to deliver drugs with antimicrobial activity (Otvos 2002; Scocchi et al. 2011) (Table 3). For example, based on the penetration ability of native PrAMP, designed pyrrhocoricin analogues conjugated with class I major histocompatibility complex epitope successfully passed through bacterial and mammalian cells (Otvos 2002; Otvos et al. 2004). Furthermore, because of the dual function of cell permeability and antimicrobial action, bactenecin 7 or its fragments might represent a novel vector to deliver protein and phosphorescent oxygen-sensitive probe into various mammalian cells (Dmitriev et al. 2010; Sadler et al. 2002). Similarly, the PrAMP, PR39, as a complex with siRNA followed by translocation into breast cancer cells, was able to inhibit cell invasion and immigration (Tian et al. 2012). Furthermore, a recent report showed that several PrAMPs, including oncocin, apidaecin (Api137), drosocin and drosocin (Pro5Hyp), could cross the BBB to selectively target brain cells (Stalmans et al. 2014). This indicated the PrAMPs would not only be potential therapeutics for cerebral infections, but also novel potential carriers for brain drug delivery.

Mechanism resistance of PrAMPs

Even though AMPs have significant potential as alternatives to current small molecule antibiotics (Marr et al. 2006), there are reports of development of bacterial resistance against them. They may be due to proteolytic cleavage, external inactive-molecules, specific exporters and electrostatic repulsion (Kraus and Peschel 2006). However, compared with other cationic AMPs, the designed PrAMP, A3-APO, displayed considerably less propensity to induce antimicrobial resistance or genetic alteration within its intracellular target, which highlighted its potential as an alternative to replace antibiotics (Cassone et al. 2009). In general, PrAMPs entered the bacteria through the inner membrane *via* the translocation of permease/transporter-mediated uptake mechanism involved membrane protein SbmA (Runti et al. 2013). However, partial antimicrobial resistance of PrAMPs has been observed with co-cultured *Escherichia coli* mutants with the deletion of *SbmA* gene (Narayanan et al. 2014). This indicates a potential induced-resistance

mechanism and the need to further develop PrAMPs with multiple targets to better combat antibiotic resistance.

Summary

In the fight against multi-drug resistant microbes, PrAMPs display potent activity when supplementing conventional antibiotics. Even though mutant *Escherichia coli* lacking the *SbmA* gene have shown the onset of resistance to PrAMPs, these peptides showed various modes of action against microbes. Furthermore, their mechanism shifts from non-disruption to membrane damage depending on concentration and can modulate the immunity system and affect different potential intracellular targets. This in turn could lead to development of more effective antimicrobial alternatives. In addition to their antimicrobial activities, PrAMPs also display the ability to penetrate cell membranes and to cross the BBB, which could lead to a new strategy for drug delivery as a potential drug carrier.

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References

Agerberth B, Lee JY, Bergman T, Carlquist M, Boman HG, Mutt V, Jornvall H (1991) Amino acid sequence of PR-39. Isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides *Eur J Biochem* 202:849-854

Anbanandam A, Albarado DC, Tirziu DC, Simons M, Veeraraghavan S (2008) Molecular basis for proline- and arginine-rich peptide inhibition of proteasome *J Mol Biol* 384:219-227

Avitabile C, D'Andrea LD, Romanelli A (2014) Circular Dichroism studies on the interactions of antimicrobial peptides with bacterial cells *Sci Rep* 4

Bahar A, Ren D (2013) Antimicrobial peptides *Pharmaceuticals* 6:1543-1575

Baumann T, Kämpfer U, Schürch S, Schaller J, Largiadèr C, Nentwig W, Kuhn-Nentwig L (2010) Ctenidins: antimicrobial glycine-rich peptides from the hemocytes of the spider Cupiennius salei *Cell Mol Life Sci* 67:2787-2798

Bencivengo A-M, Cudic M, Hoffmann R, Otvos L, Jr. (2001) The efficacy of the antibacterial peptide, pyrrhocoricin, is finely regulated by its amino acid residues and active domains *Lett Pept Sci* 8:201-209

Berthold N, Hoffmann R (2014) Cellular uptake of apidaecin 1b and related analogs in Gram-negative bacteria reveals novel antibacterial mechanism for proline-rich antimicrobial peptides *Protein Pept Lett* 21:391-398

Boman HG, Agerberth B, Boman A (1993) Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine *Infect Immun* 61:2978-2984

Brogden KA (2005) Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* 3:238-250

Brötz H, Bierbaum G, Leopold K, Reynolds PE, Sahl H-G (1998) The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II *Antimicrob Agents Chemother* 42:154-160

Bulet P, Dimarcq JL, Hetru C, Lagueux M, Charlet M, Hegy G, Van Dorsselaer A, Hoffmann JA (1993) A novel inducible antibacterial peptide of *Drosophila* carries an O-glycosylated substitution *J Biol Chem* 268:14893-14897

Bulet P, Hetru C, Dimarcq JL, Hoffmann D (1999) Antimicrobial peptides in insects; structure and function *Dev Comp Immunol* 23:329-344

Butler MS, Blaskovich MA, Cooper MA (2013) Antibiotics in the clinical pipeline in 2013 *J Antibiot (Tokyo)* 66:571-591

Cannon B (2014) Microbiology: Resistance fighters *Nature* 509:S6-S8

Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis *Nat Med* 6:389-395

Cassone M, Frith N, Vogiatzi P, Wade JD, Otvos L (2009) Induced resistance to the designer proline-rich antimicrobial peptide A3-APO does not iInvolve changes in the intracellular target DnaK *Int J Pept Res Ther* 15:121-128

Cassone M, Otvos L (2010) Synergy among antibacterial peptides and between peptides and small-molecule antibiotics *Expert Rev Anti Infect Ther* 8:703-716

Casteels P, Ampe C, Jacobs F, Vaeck M, Tempst P (1989) Apidaecins: Antibacterial peptides from honeybees *The EMBO journal* 8:2387

Chan DI, Prenner EJ, Vogel HJ (2006) Tryptophan- and arginine-rich antimicrobial peptides: Structures and mechanisms of action *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1758:1184-1202

Chen W, Luo L (2009) Classification of antimicrobial peptide using diversity measure with quadratic discriminant analysis *J Microbiol Methods* 78:94-96

Chesnokova LS, Slepenkov SV, Witt SN (2004) The insect antimicrobial peptide, l-pyrrhocoricin, binds to and stimulates the ATPase activity of both wild-type and lidless DnaK *FEBS Lett* 565:65-69

Chitnis SN, Prasad KN, Bhargava PM (1987) Bacteriolytic activity of seminalplasmin *J Gen Microbiol* 133:1265-1271

Chitnis SN, Prasad KSN, Bhargava PM (1990) Isolation and characterization of autolysis-defective mutants of *Escherichia Coli* that are resistant to the lytic activity of seminalplasmin *J Gen Microbiol* 136:463-469

Cho JH, Park CB, Yoon YG, Kim SC (1998) Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization *Biochim Biophys Acta* 1408:67-76

Cully M (2014) Public health: The politics of antibiotics *Nature* 509:S16-S17

Dagan A, Efron L, Gaidukov L, Mor A, Ginsburg H (2002) *In vitro* antiplasmodium effects of dermaseptin S4 derivatives *Antimicrob Agents Chemother* 46:1059-1066

de Souza Cândido E, e Silva Cardoso MH, Sousa DA, Viana JC, de Oliveira-Júnior NG, Miranda V, Franco OL (2014) The use of versatile plant antimicrobial peptides in agribusiness and human health *Peptides* 55:65-78

Delgado MA, Rintoul MaR, Farı´as RN, Salomón RA (2001) *Escherichia coli* RNA polymerase is the target of the cyclopeptide antibiotic microcin J25 *J Bacteriol* 183:4543-4550

Destoumieux D, Munoz M, Bulet P, Bachere E (2000) Penaeidins, a family of antimicrobial peptides from penaeid shrimp (*Crustacea, Decapoda*) *Cell Mol Life Sci* 57:1260-1271

Dimarcq J-L, Bulet P, Hetru C, Hoffmann J (1998) Cysteine-rich antimicrobial peptides in invertebrates *Peptide Science* 47:465-477

Dmitriev RI, Ropiak HM, Yashunsky DV, Ponomarev GV, Zhdanov AV, Papkovsky DB (2010) Bactenecin 7 peptide fragment as a tool for intracellular delivery of a phosphorescent oxygen sensor *FEBS J* 277:4651-4661

Dong N, Ma Q, Shan A, Lv Y, Hu W, Gu Y, Li Y (2012) Strand Length-Dependent Antimicrobial Activity and Membrane-Active Mechanism of Arginine- and Valine-Rich β-Hairpin-Like Antimicrobial Peptides *Antimicrob Agents Chemother* 56:2994-3003

Fernandez DI, Gehman JD, Separovic F (2009) Membrane interactions of antimicrobial peptides from Australian frogs *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1788:1630-1638

Fernández-Vidal M, Jayasinghe S, Ladokhin AS, White SH (2007) Folding amphipathic helices into membranes: Amphiphilicity trumps hydrophobicity *J Mol Biol* 370:459-470

Fox JL (2013) Antimicrobial peptides stage a comeback *Nat Biotechnol* 31:379-382

Gaczynska M, Osmulski PA, Gao Y, Post MJ, Simons M (2003) Proline- and arginine-rich peptides constitute a novel class of allosteric inhibitors of proteasome activity *Biochemistry (Mosc)* 42:8663-8670

Gallo RL, Ono M, Povsic T, Page C, Eriksson E, Klagsbrun M, Bernfield M (1994) Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds *Proc Natl Acad Sci U S A* 91:11035-11039

Gammon K (2014) Drug discovery: Leaving no stone unturned *Nature* 509:S10-S12

Gazit E, Boman A, Boman HG, Shai Y (1995) Interaction of the mammalian antibacterial peptide cecropin P1 with phospholipid vesicles *Biochemistry (Mosc)* 34:11479-11488

Gennaro R, Skerlavaj B, Romeo D (1989) Purification, composition, and activity of two bactenecins, antibacterial peptides of bovine neutrophils *Infect Immun* 57:3142-3146

Gennaro R, Zanetti M, Benincasa M, Podda E, Miani M (2002) Pro-rich antimicrobial peptides from animals: Structure, biological functions and mechanism of action *Curr Pharm Des* 8:763-778

Ghosh JK, Shaool D, Guillaud P, Cicéron L, Mazier D, Kustanovich I, Shai Y, Mor A (1997) Selective cytotoxicity of dermaseptin S3 toward intraerythrocytic plasmodium falciparum and the underlying molecular basis *J Biol Chem* 272:31609-31616

Giuliani A, Pirri G, Nicoletto S (2007) Antimicrobial peptides: An overview of a promising class of therapeutics *Cent Eur J Biol* 2:1-33

Hallock KJ, Lee D-K, Ramamoorthy A (2003) MSI-78, an analogue of the magainin antimicrobial peptides, disrupts lipid bilayer structure *via* positive curvature strain *Biophys J* 84:3052-3060

Hancock REW (1997) Peptide antibiotics *Lancet* 349:418-422

Hancock REW, Nijnik A, Philpott DJ (2012) Modulating immunity as a therapy for bacterial infections *Nat Rev Micro* 10:243-254

He K, Ludtke SJ, Worcester DL, Huang HW (1996) Neutron scattering in the plane of membranes: Structure of alamethicin pores *Biophys J* 70:2659-2666

Hede K (2014) Antibiotic resistance: An infectious arms race *Nature* 509:S2-S3

Henzler Wildman KA, Lee D-K, Ramamoorthy A (2003) Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37 *Biochemistry (Mosc)* 42:6545-6558

Hernandez-Gordillo V, Geisler I, Chmielewski J (2014) Dimeric unnatural polyproline-rich peptides with enhanced antibacterial activity *Bioorg Med Chem Lett* 24:556-559

Hilchie AL, Wuerth K, Hancock REW (2013) Immune modulation by multifaceted cationic host defense (antimicrobial) peptides *Nat Chem Biol* 9:761-768

Hsueh P-R (2012) Study for monitoring antimicrobial resistance trends (SMART) in the Asia-Pacific region, 2002–2010 *Int J Antimicrob Agents* 40:S1-S3

Ilić N, Novković M, Guida F, Xhindoli D, Benincasa M, Tossi A, Juretić D (2013) Selective antimicrobial activity and mode of action of adepantins, glycine-rich peptide antibiotics based on anuran antimicrobial peptide sequences *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1828:1004-1012

Jenssen H, Hamill P, Hancock REW (2006) Peptide antimicrobial agents *Clin Microbiol Rev* 19:491-511

Kanthor R (2014) Diagnostics: Detection drives defence *Nature* 509:S14-S15

Kavanagh K, Dowd S (2004) Histatins: Antimicrobial peptides with therapeutic potential *J Pharm Pharmacol* 56:285-289

Kraus D, Peschel A (2006) Molecular mechanisms of bacterial resistance to antimicrobial peptides *Curr Top Microbiol Immunol* 306:231-250

Lalatsa A, Schatzlein AG, Uchegbu IF (2014) Strategies to deliver peptide drugs to the brain *Mol Pharm* 11:1081-1093

Lehrer R, Barton A, Daher K, Harwig S, Ganz T, Selsted M (1989) Interaction of human defensins with *Escherichia coli* mechanism of bactericidal activity *J Clin Invest* 84:553

Li CY, Song YL (2010) Proline-rich domain of penaeidin molecule exhibits autocrine feature by attracting penaeidin-positive granulocytes toward the wound-induced inflammatory site *Fish Shellfish Immunol* 29:1044-1052

Lijuan Zhang TJF (2006) Antimicrobial peptides: Therapeutic potential *Expert Opin Pharmacother* 7:653-663

Ludtke SJ, He K, Heller WT, Harroun TA, Yang L, Huang HW (1996) Membrane pores induced by magainin *Biochemistry (Mosc)* 35:13723-13728

Marr AK, Gooderham WJ, Hancock REW (2006) Antibacterial peptides for therapeutic use: obstacles and realistic outlook *Curr Opin Pharmacol* 6:468-472

Matsuzaki K (1999) Why and how are peptide–lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1462:1-10

Matsuzaki K, Murase O, Fujii N, Miyajima K (1996) An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation *Biochemistry (Mosc)* 35:11361-11368

Matsuzaki K, Sugishita K-i, Ishibe N, Ueha M, Nakata S, Miyajima K, Epand RM (1998) Relationship of membrane curvature to the formation of pores by magainin 2 *Biochemistry (Mosc)* 37:11856-11863

Naito A, Nagao T, Norisada K, Mizuno T, Tuzi S, Saitô H (2000) Conformation and dynamics of melittin bound to magnetically oriented lipid bilayers by solid-state 31P and 13C NMR spectroscopy *Biophys J* 78:2405-2417

Narayanan S, Modak JK, Ryan CS, Garcia-Bustos J, Davies JK, Roujeinikova A (2014) Mechanism of *Escherichia coli* resistance to pyrrhocoricin *Antimicrob Agents Chemother*

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* 341:501-513

Ostorhazi E, Holub MC, Rozgonyi F, Harmos F, Cassone M, Wade JD, Otvos L, Jr. (2011) Broad-spectrum antimicrobial efficacy of peptide A3-APO in mouse models of multidrug-resistant wound and lung infections cannot be explained by in vitro activity against the pathogens involved *Int J Antimicrob Agents* 37:480-484

Otvos L (2000) Antibacterial peptides isolated from insects *J Pept Sci* 6:497-511

Otvos L (2002) The short proline-rich antibacterial peptide family *Cell Mol Life Sci* 59:1138-1150

Otvos L (2005) Antibacterial peptides and proteins with multiple cellular targets *J Pept Sci* 11:697-706

Otvos L, Cudic M, Chua BY, Deliyannis G, Jackson DC (2004) An insect antibacterial peptide-based drug delivery system *Mol Pharm* 1:220-232

Otvos L, Flick-Smith H, Fox M, Ostorhazi E, Dawson RM, Wade JD (2014) The designer proline-rich antibacterial peptide A3-APO prevents bacillus anthracis mortality by deactivating bacterial toxins *Protein Pept Lett* 21:374-381

Otvos L, O I, Rogers ME, Consolvo PJ, Condie BA, Lovas S, Bulet P, Blaszczyk-Thurin M (2000) Interaction between heat shock proteins and antimicrobial peptides *Biochemistry (Mosc)* 39:14150-14159

Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock REW (2002) Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli Antimicrob Agents Chemother* 46:605-614

Paulsen VS, Blencke H-M, Benincasa M, Haug T, Eksteen JJ, Styrvold OB, Scocchi M, Stensvåg K (2013) Structure-activity relationships of the antimicrobial peptide arasin 1 - and mode of action studies of the N-terminal, proline-rich region *PLoS One* 8:e53326

Pelillo C, Benincasa M, Scocchi M, Gennaro R, Tossi A, Pacor S (2014) Cellular internalization and cytotoxicity of the antimicrobial proline-rich peptide Bac7(1-35) in monocytes/macrophages, and its activity against phagocytosed Salmonella typhimurium *Protein Pept Lett* 21:382-390

Peters BM, Shirtliff ME, Jabra-Rizk MA (2010) Antimicrobial peptides: Primeval molecules or future drugs? *PLoS Pathog* 6:e1001067

Podda E, Benincasa M, Pacor S, Micali F, Mattiuzzo M, Gennaro R, Scocchi M (2006) Dual mode of action of Bac7, a proline-rich antibacterial peptide *Biochimica et Biophysica Acta (BBA) - General Subjects* 1760:1732-1740

Pouny Y, Rapaport D, Mor A, Nicolas P, Shai Y (1992) Interaction of antimicrobial dermaseptin and its fluorescently labeled analogs with phospholipid membranes *Biochemistry (Mosc)* 31:12416-12423

Powers J-PS, Hancock REW (2003) The relationship between peptide structure and antibacterial activity *Peptides* 24:1681-1691

Pushpanathan M, Gunasekaran P, Rajendhran J (2013) Antimicrobial peptides: Versatile biological properties *Int J Pept* 2013:15

Rappocciolo E (2004) Antimicrobial peptides as carriers of drugs *Drug Discov Today* 9:470

Reddy KVR, Yedery RD, Aranha C (2004) Antimicrobial peptides: Premises and promises *Int J Antimicrob Agents* 24:536-547

Rolland JL, Abdelouahab M, Dupont J, Lefevre F, Bachere E, Romestand B (2010) Stylicins, a new family of antimicrobial peptides from the Pacific blue shrimp *Litopenaeus stylirostris Mol Immunol* 47:1269-1277

Rozgonyi F, Szabo D, Kocsis B, Ostorhazi E, Abbadessa G, Cassone M, Wade JD, Otvos L (2009) The antibacterial effect of a proline-rich antibacterial peptide A3-APO *Curr Med Chem* 16:3996-4002

Runti G, Lopez Ruiz MdC, Stoilova T, Hussain R, Jennions M, Choudhury HG, Benincasa M, Gennaro R, Beis K, Scocchi M (2013) Functional characterization of SbmA, a bacterial inner membrane transporter required for importing the antimicrobial peptide Bac7(1-35) *J Bacteriol* 195:5343-5351

Sadler K, Eom KD, Yang J-L, Dimitrova Y, Tam JP (2002) Translocating proline-rich peptides from the antimicrobial peptide Bactenecin 7 *Biochemistry (Mosc)* 41:14150-14157

Salomón RA, Farías RN (1992) Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli J Bacteriol* 174:7428-7435

Scheit KH, Reddy ESP, Bhargava PM (1979) Seminalplasmin is a potent inhibitor of *E. coli* RNA polymerase *in vitro Nature* 279:728-731

Scocchi M, Tossi A, Gennaro R (2011) Proline-rich antimicrobial peptides: Converging to a non-lytic mechanism of action *Cell Mol Life Sci* 68:2317-2330

Shai Y (1995) Molecular recognition between membrane-spanning polypeptides *Trends Biochem Sci* 20:460-464

Shai Y (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membrane-lytic peptides *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1462:55-70

Shamova O, Brogden KA, Zhao C, Nguyen T, Kokryakov VN, Lehrer RI (1999) Purification and properties of proline-rich antimicrobial peptides from sheep and goat leukocytes *Infect Immun* 67:4106-4111

Shen X, Ye G, Cheng X, Yu C, Altosaar I, Hu C (2010) Characterization of an abaecin-like antimicrobial peptide identified from a Pteromalus puparum cDNA clone *J Invertebr Pathol* 105:24-29

Shi J, Ross CR, Chengappa MM, Sylte MJ, McVey DS, Blecha F (1996) Antibacterial activity of a synthetic peptide (PR-26) derived from PR-39, a proline-arginine-rich neutrophil antimicrobial peptide *Antimicrob Agents Chemother* 40:115-121

Smith R, Separovic F, Bennett FC, Cornell BA (1992) Melittin-induced changes in lipid multilayers. A solid-state NMR study *Biophys J* 63:469-474

Splith K, Neundorf I (2011) Antimicrobial peptides with cell-penetrating peptide properties and vice versa *Eur Biophys J* 40:387-397

Stalmans S, Wynendaele E, Bracke N, Knappe D, Hoffmann R, Peremans K, Polis I, Burvenich C, De Spiegeleer B (2014) Blood-brain barrier transport of short proline-rich antimicrobial peptides *Protein Pept Lett* 21:399-406

Stensvag K, Haug T, Sperstad SV, Rekdal O, Indrevoll B, Styrvold OB (2008) Arasin 1, a proline-arginine-rich antimicrobial peptide isolated from the spider crab, *Hyas araneus Dev Comp Immunol* 32:275-285

Stewart KM, Horton KL, Kelley SO (2008) Cell-penetrating peptides as delivery vehicles for biology and medicine *Org Biomol Chem* 6:2242-2255

Subbalakshmi C, Sitaram N (1998) Mechanism of antimicrobial action of indolicidin *FEMS Microbiol Lett* 160:91-96

Szabo D, Ostorhazi E, Binas A, Rozgonyi F, Kocsis B, Cassone M, Wade JD, Nolte O, Otvos L, Jr. (2010) The designer proline-rich antibacterial peptide A3-APO is effective against systemic *Escherichia coli* infections in different mouse models *Int J Antimicrob Agents* 35:357-361

Tian W, Li B, Zhang X, Dang W, Wang X, Tang H, Wang L, Cao H, Chen T (2012) Suppression of tumor invasion and migration in breast cancer cells following delivery of siRNA against Stat3 with the antimicrobial peptide PR39 *Oncol Rep* 28:1362-1368

Végh AG, Nagy K, Bálint Z, Kerényi Á, Rákhely G, Váró G, Szegletes Z (2011) Effect of antimicrobial peptide-amide: indolicidin on biological membranes *J Biomed Biotechnol* 2011

Wilson SS, Wiens ME, Smith JG (2013) Antiviral mechanisms of human defensins *J Mol Biol* 425:4965-4980

Wong H, Bowie JH, Carver JA (1997) The solution structure and activity of caerin 1.1, an antimicrobial peptide from the Australian green tree frog, *Litoria Splendida Eur J Biochem* 247:545-557

Wu M, Maier E, Benz R, Hancock REW (1999) Mechanism of interaction of different classes of cationic antimicrobial peptides with planar bilayers and with the cytoplasmic membrane of *Escherichia coli Biochemistry (Mosc)* 38:7235-7242

Yamaguchi S, Hong T, Waring A, Lehrer RI, Hong M (2002) Solid-state NMR investigations of peptide–lipid interaction and orientation of a β-sheet antimicrobial peptide, protegrin *Biochemistry (Mosc)* 41:9852-9862

Yamaguchi S, Huster D, Waring A, Lehrer RI, Kearney W, Tack BF, Hong M (2001) Orientation and dynamics of an antimicrobial peptide in the lipid bilayer by solid-state NMR spectroscopy *Biophys J* 81:2203-2214

Yang L, Harroun TA, Weiss TM, Ding L, Huang HW (2001) Barrel-stave model or toroidal model? A case study on melittin pores *Biophys J* 81:1475-1485

Yang L, Weiss TM, Lehrer RI, Huang HW (2000) Crystallization of antimicrobial pores in membranes: Mgainin and ptegrin *Biophys J* 79:2002-2009

Yu P-L, Cross ML, Haverkamp RG (2010) Antimicrobial and immunomodulatory activities of an ovine proline/arginine-rich cathelicidin *Int J Antimicrob Agents* 35:288-291

Zahn M, Berthold N, Kieslich B, Knappe D, Hoffmann R, Sträter N (2013) Structural studies on the forward and reverse binding modes of peptides to the chaperone DnaK *J Mol Biol* 425:2463-2479

Zahn M, Kieslich B, Berthold N, Knappe D, Hoffmann R, Strater N (2014) Structural identification of DnaK binding dites within bovine and sheep Bactenecin Bac7 *Protein Pept Lett* 21:407-412

Zasloff M (2002) Antimicrobial peptides of multicellular organisms *Nature* 415:389-395

Zhang R, Eggleston K, Rotimi V, Zeckhauser RJ (2006) Antibiotic resistance as a global threat: Evidence from China, Kuwait and the United States *Global Health* 2:6

Zhou Y, Chen WN (2011) iTRAQ-Coupled 2-D LC-MS/MS analysis of membrane protein profile in *Escherichia coli* incubated with apidaecin IB *PLoS One* 6:e20442

Zhu Y-G, Johnson TA, Su J-Q, Qiao M, Guo G-X, Stedtfeld RD, Hashsham SA, Tiedje JM (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms *Proc Natl Acad Sci U S A* 110:3435-3440

Table 1. Examples of AMPs with pore forming or non-lytic mechanism against microbes

pore formation mechanism	barrel stave model	alamethicin (He et al. 1996; Yang et al. 2001)
	carpet pore formation	dermaseptin S (Dagan et al. 2002; Ghosh et al. 1997), cecropin (Gazit et al. 1995; Shai 1995), melittin (Naito et al. 2000), aurein 1.2 (Fernandez et al. 2009), caerin 1.1 (Wong et al. 1997), ovispirin (Yamaguchi et al. 2001) and LL37 (Oren et al. 1999)
	toroidal model	magainin 2 (Ludtke et al. 1996; Matsuzaki et al. 1996), protegrin-1 (Yamaguchi et al. 2002), melittin (Smith et al. 1992; Yang et al. 2001), LL-37 (Henzler Wildman et al. 2003), MSI-78 (Hallock et al. 2003)
	aggregate model	dermaseptin (Pouny et al. 1992), magainin 2 (Matsuzaki et al. 1996)
non-lytic mechanism	inhibition of macromolecular synthesis	indolicin (Subbalakshmi and Sitaram 1998), PR-39 (Boman et al. 1993), pleurocidin (Patrzykat et al. 2002), seminalplasmin (Scheit et al. 1979), tPMP-1 (Lehrer et al. 1989), aHNP-1 (Lehrer et al. 1989), microcin J25 (Delgado et al. 2001)
	inhibition of cell-wall/membrane formation	lantibiotic mersacidin (Brötz et al. 1998), PR-39 (Shi et al. 1996), PR-26 (Shi et al. 1996), indolicin (Subbalakshmi and Sitaram 1998), microcin J25 (Salomón and Farías 1992)
	inhibition of metabolic function	histatins (Kavanagh and Dowd 2004), pyrrhocoricin (Bencivengo et al. 2001; Chesnokova et al. 2004), drosocin (Chesnokova et al. 2004), apidaecin (Casteels et al. 1989), aiptercin (Chitnis et al. 1987; Chitnis et al. 1990)

Table 2. Proline-rich AMPs modulation of the immune system *via* cytokine activity or angiogenesis or proteasome inhibition.

cytokine activity	penaeidin	acts as cytokine (Li and Song 2010)
	Bac7 (1-35)	activates defence response (Pelillo et al. 2014)
	cathelicidin OaBac5mini	decreases the production of cytokine interleukin-12 (Yu et al. 2010)
	A3-APO	cytokines interleukin-4/interleukin-10 (Ostorhazi et al. 2011; Otvos et al. 2014)
angiogenesis	PR39	proteasome inhibition/syndecans induction/angiogenesis stimulation (Anbanandam et al. 2008; Gaczynska et al. 2003; Gallo et al. 1994; Gennaro et al. 2002)

Table 3 Proline-rich AMPs able to penetrate cell membranes and cross blood brain barrier as novel potential carriers for drug delivery.

Cell Penetrating Peptides (CPP)	pyrrhocoricin	delivery of peptidic/epitope based cargo (Otvos et al. 2004; Rappocciolo 2004)
	bactenecin 7	carrier for protein cargo and phosphorescent oxygen sensor (Dmitriev et al. 2010; Sadler et al. 2002)
	PR39	delivery of siRNA into 4T1 cells(Tian et al. 2012)
Blood Brain Barrier (BBB) crossing	oncocin	mostly destined for endothelial cells (Stalmans et al. 2014)
	apidaecin (Api137)	mainly trapped in the brain parenchyma (Stalmans et al. 2014)
	drosocin	
	drosocin (Pro5Hyp)	

FIGURE LEGENDS

Figure 1. **The period for resistance to emerge for conventional antibiotics after their release**. These includes penicillin, tetracycline, erythromycin, methicillin, gentamicin, vancomycin, imipenem, ceftazidime, levofloxacin, linezolid, ceftaroline. This figure has been modified with permission from Hede (2014).

Figure 2. **Lytic and non-lytic mechanisms of AMPs**: A) barrel stave model, B) carpet pore formation, C) toroidal model, D) aggregate model; inhibition of macromolecular synthesis, inhibition of cell-wall/membrane formation, and inhibition of metabolice function.

FIGURE 1.

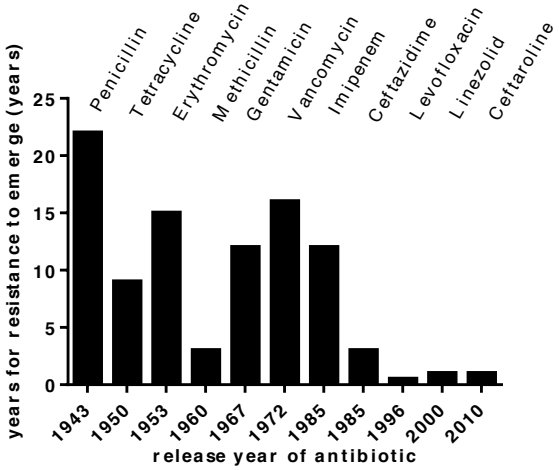
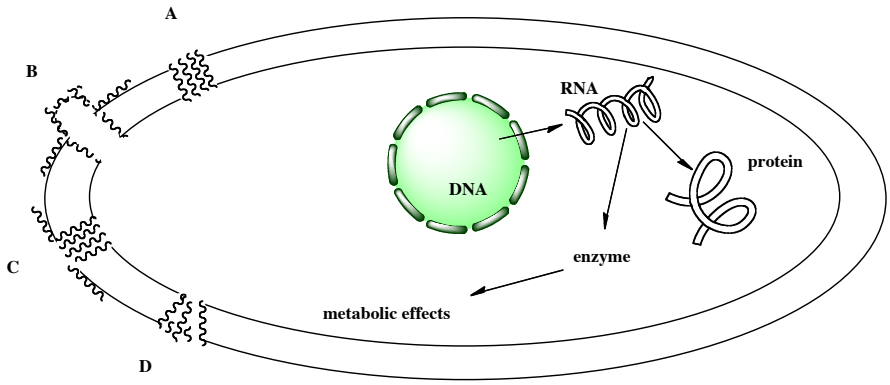

Penicillin
Tetracycline
Erythromycin
Methicillin
Gentamicin
Vancomycin
Imipenem
Ceftazidime
Levofloxacin
Linezolid
Ceftaroline
years for resistance to emerge (years)
25
20
15
10
5
0
1943
1950
1953
1960
1967
1972
1985
1985
1996
2000
2010
release year of antibiotic

FIGURE 2.



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