Synergy and duality in peptide antibiotic mechanisms

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The molecular mechanisms by which peptide antibiotics disrupt bacterial DNA synthesis, protein biosynthesis, cell wall biosynthesis, and membrane integrity are diverse, yet historically have been understood to follow a theme of one antibiotic, one inhibitory mechanism. In the past year, mechanistic and structural studies have shown a rich diversity in peptide antibiotic mechanism. Novel secondary targeting mechanisms for peptide antibiotics have recently been discovered, and the mechanisms of peptide antibiotics involved in synergistic relationships with antibiotics and proteins have been more clearly defined. In apparent response to selective pressures, antibiotic-producing organisms have elegantly integrated multiple functions and cooperative interactions into peptide antibiotic design for the purpose of improving antimicrobial success.

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Abbreviations

EF	elongation factor
Lipid I	undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide
Lipid II	undecaprenyl-pyrophosphoryl-MurNAc-(GlcNAc)-pentapeptide
PBP	penicillin-binding protein
PE	phosphatidylethanolamine

Introduction

Peptide antibiotics are produced by bacterial, mammalian, insect, and plant organisms in defense against invasive microbial pathogens. Evolution has crafted peptide antibiotics into functionally optimized compounds with defined specificity and higher order functionality. The number and composition of peptide antibiotics are myriad, reflecting origins from ribosomal, post-translational, or non-ribosomal biosynthetic means. Likewise their modes of action are equally diverse. As our understanding of peptide antibiotic mechanisms steadily increases, two prevalent mechanistic themes are emerging: molecular synergy and functional duality. Molecular synergy is defined as the combined action of two or more antibiotics toward a single target molecule. Functional duality is defined as one molecule having dual antibiotic activity. The purpose of this review is to survey recent examples of the intriguing themes of synergy and duality in peptide antibiotic mechanism.

Synergistic peptide antibiotics

Synergism in targeting ribosomal protein biosynthesis

Thiopeptide antibiotics

Thiostrepton and micrococcin are members of the class of thiazole-containing peptide antibiotics (or thiopeptide antibiotics), which inhibit protein biosynthesis by binding to the 23S rRNA subunit of the ribosome and preventing its proper function [1•–3•]. This family of antibiotics (see Figure 1) is characterized by highly modified peptide backbones, in which thiazole, 4,2′-bisthiazole, didehydroalanine and didehydrobutyrine residues are formed from oxidative cyclization and dehydration modifications of cysteine, serine and threonine residues [4•].

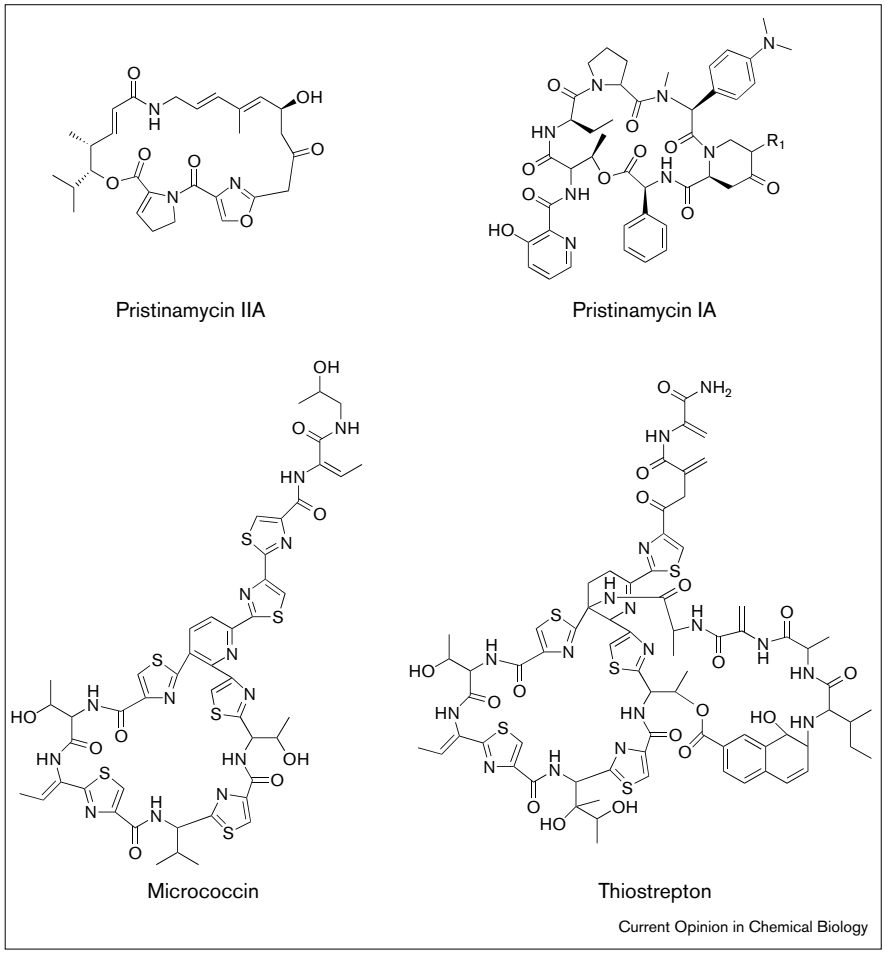
The 23S rRNA site where GTPase-driven elongation factors EF-Tu and EF-G bind is one of the most important functional regions of the ribosome. This site is characterized by two highly conserved regions of the 23S rRNA ribosomal subunit, the 17-nucleotide sarcin/ricin loop, and the 58-nucleotide domain associated with the ribosomal protein L11. Binding of the L11 carboxyl terminus to the 58-nucleotide domain of the rRNA stabilizes its folded conformation [5••,6••]. The amino-terminal domain of L11 is highly conserved, proline-rich, and functions as a molecular switch, mediating a conformational change that allows elongation factors to properly bind and propagate peptide synthesis. Thiopeptide antibiotics bind to the 23S rRNA and place a conformational constraint on protein L11, locking it into a conformation that disfavors proper binding and/or function of EF-Tu and EF-G, effectively halting protein synthesis [1•,2•].

Remarkably, thiostrepton-producing microorganisms have tailored this antibiotic not only to target a unique, functionally-important conformational change in protein L11 but also to manipulate this essential protein for the purpose of conferring cooperative, high-affinity binding and intimate specificity. Binding of thiostrepton and L11 protein together to rRNA is synergistic and cooperative and essentially irreversible [7]; however, in the absence of rRNA, thiostrepton and L11 do not associate and without L11 thiostrepton affinity for rRNA is comparatively weak [6••].

The molecular basis for this unusual synergistic relationship is rapidly becoming clearer. Resistant mutants and chemical footprinting experiments have been employed to predict the target sites of thiostrepton and micrococcin. High level resistance to thiostrepton is conferred both by mutation of the conserved amino-terminal helix of L11 at Pro22, as well as by rRNA 2′-*O*-methylation at A1067 and mutation of nucleotides A1067 and A1095. Footprinting experiments confirmed protection of these nucleotide positions with thiostrepton and micrococcin, suggesting that

Figure 1

Chemical structures of synergistic ribosome-targeting peptide antibiotics.



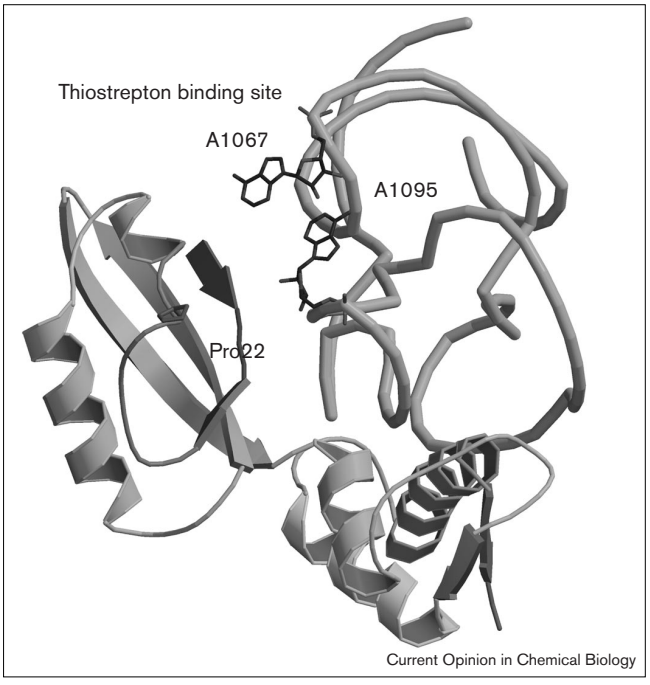
the two antibiotics bind to overlapping regions of rRNA. White and co-workers [6••] recently reported the crystal structure of the L11–rRNA complex, which confirmed the hypothesis that A1067, A1089 of the rRNA and the amino-terminal proline-rich helix of L11 are adjacent and suggests that the exposed cleft between the three juxtaposed strands is the candidate thiostrepton-binding site (Figure 2). From the structure of the L11–rRNA complex, synergistic binding could be explained by initial displacement of a loosely-associated amino-terminal helix of L11 by thiostrepton followed by back-binding of the displaced helix onto the rRNA–antibiotic complex, locking in the tightly-associated ternary complex. Thiopeptides also contain potentially reactive didehydroalanine residues, which could participate in covalent capture of either rRNA or L11. Confirmation of structure of the ternary complex by X-ray analysis is anxiously awaited and will, we hope, better our understanding of the molecular basis of the observed synergy and high affinity association.

Streptogramin antibiotics

The streptogramin antibiotics (also known as the synergimycins) consist of pairs of structurally unrelated peptide-derived antibiotics that act synergistically to inhibit ribosomal peptidyl transfer during bacterial protein biosynthesis [8–10]. Streptogramin type A (pristinamycin IIA, virginiamycin M_1) and type B (pristinamycin IA, virginiamycin S_1) antibiotics are macrocyclic lactone peptolides produced by *Streptomyces pristinaespiralis* and *Streptomyces virginiae*. Type A streptogramins inactivate the donor (P) and acceptor (A) sites of the peptidyl transferase region of the 23S rRNA subunit by blocking two of the peptide chain elongation steps: aminoacyl–tRNA binding to the A site; and peptide bond formation with peptidyl–tRNA at the P site. The type B streptogramins inhibit peptide bond formation by binding to the 23S rRNA peptidyl transferase loop, forcing the release of incomplete peptide chains [9,10]. This mode of action is similar to that of the 14-member macrolide family of antibiotics (which includes erythromycin) and also to that of the lincosamides, and suggests that streptogramin B antibiotics share overlapping binding sites with these structurally unrelated antibiotics [9].

Separately, streptogramins A and B are bacteriostatic, yet when administered in combination *in vivo* the antibiotics

Figure 2



Three-dimensional structure of the L11–rRNA protein–RNA complex, a fragment of the 23S ribosomal subunit involved in protein translation. The conserved portion of the rRNA is the target of the thiostrepton/micrococcin family of thiopeptide antibiotics. Resistance to thiostrepton is conferred by mutations in L11 at Pro22, or in the rRNA at positions A1067 or A1095. From the crystal structure, the close proximity of these residues suggests that thiostrepton binds in the cleft formed between the amino-terminal helix and the two juxtaposed RNA loops.

are bactericidal and their inhibitory action is synergistic. Binding of streptogramin A antibiotics to the ribosome ($K_a = 2.5 \times 10^6$ M) increases the affinity for streptogramin B up to 40-fold and decreases the affinity for other macrolide antibiotics [8,11], whereas dual antibiotic binding (A and B bound) is essentially irreversible [9]. It is believed that the binding of type A antibiotics alters the conformation of the rRNA and exposes a high affinity binding site for the type B antibiotic [9].

This year, Porse and Garrett [12•] have shed light on the molecular target of the synergetic streptogramin antibiotics. Resistance to both antibiotics has been linked to RNA A2058 mutation or N-6 base methylation. Photoaffinity experiments indicate that streptogramin B cross-links directly to A2503/U2504 rRNA and induces an internal ribosomal cross-link involving bases G2061/A2062 (*Escherichia coli* nomenclature) [13]. Secondly, using *in vivo* and *in vitro* footprinting experiments with streptogramin A and B bound to wildtype and mutant haloarchael and bacterial ribosomes, Porse and coworkers [13] have determined that streptogramins A and B bind to nucleotides A2058, A2059, and A2503 of the peptidyl transferase loop of the 23S rRNA. Both antibiotics have the same RNA binding site (as shown by their overlapping footprints), suggesting that a unique heterodimeric antibiotic complex forms upon contact with RNA that juxtaposes regions 2058–2062 with 2503–2506, simultaneously bridging the two adjacent strands of the 23S rRNA.

Synergism in membrane permeabilization

As a defense mechanism against microbial pathogens, insects, mammals, amphibians, and plants produce antimicrobial polypeptides such as the defensins, cecropins, magainins, and the type A lantibiotics [14•–16•,17,18•,19•]. Antimicrobial peptides in these classes are produced ribosomally, and in some cases undergo further post-translational modifications that are essential for export and optimal activity. Typically cationic, peptides in these classes generally act by interacting with anionic membrane phospholipids causing pore formation, which leads to disruption of the proton motive force, cell metabolite leakage, and cellular lysis. Within these classes (Table 1) a common theme of functional synergism between membrane targeting peptide antibiotics is rapidly emerging.

Magainins

Magainin 2 and PGLa are members of the magainin family of cationic antimicrobial peptides produced by *Xenopus laevis*. Separately, the peptides exhibit broad spectrum antibiotic activity without significant toxicity or hemolysis in humans and are therefore promising therapeutic candidates [20]. Both peptides are unstructured in aqueous solution, yet adopt an amphipathic helical structure upon exposure to membrane phospholipids. By a mechanism that involves a planar orientation of the amphipathic helices on the membrane surface [21–23], both magainin 2 and PGLa exert their antimicrobial activity by forming pores in the cytoplasmic membrane, causing membrane disruption and cell lysis [24].

When administered in combination, magainin 2 and PGLa show a marked functional synergism in bacteria, tumor cells, and artificial membranes. The two peptides exhibit a sigmoidal dependence of activity on concentration, indicating that they act in a cooperative manner. Matsuzaki *et al.* [25•] determined that magainin 2 and PGLa form a complex with a 1:1 stoichiometry ($\Delta G_{assoc} = -15$kJ/mol) and suggest that the two peptides associate into a heterosupramolecular peptide–lipid pore complex upon contact with the membrane phospholipids. In solution, magainin 2 and PGLa do not associate, nor do they posess any helical structure as assessed by circular dichroism. However, in the presence of membrane-mimicking large unilamellar vesicles, and when separate, both peptides gained significant helical structure, and a 1:1 mixture of the peptides adopted helical structure halfway between that of the individual peptides (PGLa > 1:1 mixture > magainin 2). The pore formed by magainin 2 is relatively long-lived, yet the rate of its formation is slow. In contrast, PGLa forms pores rapidly, yet they are relatively unstable. When presented as a 1:1 mixture to membranes, however, magainin 2 and PGLa form a complex that retains

Table 1

Sequences of synergistic pairs of membrane-active antimicrobial peptides.

Peptide antibiotic pairs		Sequence of mature peptide in single-letter code for amino acids*	References
1a	Acidocin J1132α	NPKVAHCASQIGRSTAWGAVSGA...(partial sequence)	[28]
1b	Acidocin J1132β	GPKVAHCASQIGRSTAWGAVSGA...(partial sequence)	
2a	Brochocin C BrcA	YSSKDCLKDIGKGIGAGTVAGAAGGGLAAGLGAIPGAFVGAHFGVIGGSAACIGGLLGN	[37]
2b	Brochocin C BrcB	KINWGNVGGSCVGGAVIGGALGGLGGAGGGCITGAIGSIWDQW	
3a	Cytolysin $CylL_S$	TTPACFTIGLGVGALFSAKFC	[38]
3b	Cytolysin $CylL_L$	TTPVCAVAATAAASSAACGWVGGGIFTGVTVVVSLKHC	
4a	Enterocin L50A	MGAIAKLVAKFGWPIVKKYYKQIMQFIGEGWAINKIIEWIKKHI	[39,40]
4b	Enterocin L50B	MGAIAKLVTKFGWPLIKKFYKQIMQFIGQGWTIDQIEKWLKRH	
5a	Lacticin F LafX	NRWGDTVLSAASGAGTGIKACKSFGPWGMAICGVGGAAIGGYFGYTHN	[33]
5b	Lacticin F LafA	RNNWQTNVGGAVGSAMIGATVGGTICGPACAVAGAHYLPILWTGVTAATGGFGKIRK	
6a	Lactococcin G α_1	GTWDDIGQGIGRVAYWVGKAMGNMSDVNQASRINRKKKH	[34,36]
6b	Lactococcin G β	KKWGWLAWVDPAYEFIKGFGKGAIKEGNKDKWKNI	
7a	Lactococcin M	IRGTGKGLAAAMVSGAAMGGAIGAFGGPVGAIMGAWGGAVGGAMKYSI	[31]
7b	Lactococcin N	MKKDEANTFKEYSSSFAIVTDEELENING	
8a	Leucocin H α	WXIGVTGAALGTGHylGVHylNVI... (partial sequence)	[30]
8b	Leucocin H β	WXAVFXNAKYMFKSQSKXVVIGFLVAS... (partial sequence)	
9a	Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS	[25•]
9b	PGLa	GMASKAGAIAGKIAKVALKAL-NH_2	
10a	Planticarin E	FNRGGYNFGKSVRHVVDAIGSVAGIRGILKSIR	[29•]
10b	Planticarin F	VFHAYSARGVRNNYKSAVGPADWVISAVRGIHG	
11a	Planticarin J	GAWKNFWSSLRKGFYDGEAGRAIRR	[29•]
11b	Planticarin K	RRSRKNGIGYAIGYAFGAVERAVLGGSRDYNK	
12a	Staphylococcin C55α	XXDhbNXFDhaLXDYWGNKGNWCTATHECMSWCK (where X = Lan or β-MeLan residues)	[41,42]
12b	Staphylococcin C55β	GDhbPLXLLGGAADhbGVIGYIXNQTXPTTACTRAC	
13a	Thermophilin 13 ThmA	YSGKDCLKDMGGYALAGAGSGALWGAPAGGVGALPGAFVGAHVGAIAGGFACMGGMIGNKFN	[27]
13b	Thermophilin 13 ThmB	QINWGSVVGHCIGGAIIGGAFSGGAAAGVGCLVGSGKAIINGL	

*-NH_2 denotes a modified carboxyl terminus ($CONH_2$). β-Lan, β-methyllanthionine; Dha, didehydroalanine; Dhb, didehydrobutyrine; Hyl, hydroxylysine; Lan, lanthionine.

the advantages of each — namely fast pore formation and moderate pore stability [25•].

Bacteriocins

Bacteriocins are relatively small, secreted antibiotic peptides that typically possess narrow species-specific or strain-specific antimicrobial profiles [26]. Over a dozen examples of synergistically-active pore-forming antibiotic pairs called two-peptide or two-component bacteriocins have recently been identified and their mechanisms partially characterized. Each component peptide of a two-peptide bacteriocin pair typically has little to no antibiotic activity, yet when administered in combination their antibiotic effects are dramatically increased well beyond the predicted sum of each peptide alone. Examples of recently characterized two-component bacteriocins include the following: thermophilin 13 α and β [27]; acidocin J1132 α and β [28]; planticarin E/F and planticarin J/K [29•]; leucocin H α and β [30]; lactococcin M and N [31]; lacticin 3147-1 and 3147-2 [32]; lactacin F A and X [33]; lactococcin G α and β [34–36]; brochocin A and B [37]; cytolysin $CylL_S$ and $CylL_L$ [38]; enterocin L50 A and B [39,40]; and staphylococcin C55 α and β [41,42]. Though the molecular basis of the synergetic antibiotic activities of two-peptide bacteriocins is not completely understood at this time, recent study of the lactococcin Gα/β and planticarin E/F, J/K systems has revealed some key elements of the molecular logic of synergy in these unique antibiotic peptides.

Lactic acid bacteria secrete Lactococcin G, the pore-forming activity of which depends on the synergistic action of two polypeptides, α and β [34,36]. Separately, each peptide has an IC_{50} value of >29 nM against sensitive *Lactobacillus* strains, yet when presented in a 1:1 combination [36] the IC_{50} value lowers 580-fold to 50 pM and ion-selective pores form with concomitant efflux of K^+, Na^+, or $^{86}Rb^+$ ions [35]. Both α and β peptides are cationic and amphipathic and adopt a helical structure in liposomes and trifluoroethanol [43]. Pore formation is linked to the onset of helical structure induced by membrane contact with the amphipathic helices. The α and β peptides synergistically induce α-helicity in each other. This α-helical structuring only occurs when the peptides are presented as a 1:1 mixture to predominantly anionic liposomes (dioeylphosphatidylcholine but not dioeylphosphatidylglycerol liposomes). It does not occur when the peptides are presented sequentially or when two separate peptide-liposome solutions are mixed [43]. When administered separately, each peptide binds irreversibly to the membrane and undergoes a conformational change that is nonfunctional and precludes rescue of the active pore by addition of the second component peptide.

The planticarins (E, F, J and K) are four cationic and amphipathic antibiotic peptides produced by *Lactobacillus plantarum* C11 that are active as pairs (E/F and J/K) against strains of *Lactobacillus* and *Pediococcus*

[29•]. Planticarins act by forming pores in the membranes of target cells and dissipating the transmembrane electrochemical potential and pH gradient. Planticarin E/F and J/K are, remarkably, both functionally and structurally synergistic. Against a susceptible *Lactobacillus plantarum* 965 test strain, planticarin E/F formed pores that efficiently conducted small monovalent cations but did not conduct anions. In contrast, plantaricin J/K formed ion-selective pores that selectively conducted specific anions but not cations, indicating that the two bacteriocins are secreted with complementary activity to ensure efficient killing of target bacteria [44]. Separately, each planticarin peptide adopts significant α-helical structure in the presence of liposomes or trifluoroethanol. When applied in combination to liposomes, however, the peptide pairs E/F and J/K induce additional α-helical structure in each other and their antibiotic activity sharply increases. Neither E nor F peptides could complement the activity or helical structuring of J or K. It is believed that complementary peptides interact with each other and with the membrane in a structure-inducing fashion, resulting in formation of an ion-selective heterosupramolecular lipid–peptide complex with amphipathic helical structure, and this complex functions more efficiently than either peptide alone.

Interestingly, acidocins J1132 α and β — also synergistic pore-forming antimicrobial peptides — self-assemble into >100 kDa aggregate complexes in solution [28]. As the two-peptide bacteriocins planticarin E/F, planticarin J/K, and lactococcin G α and β (and in all likelihood others listed above) require that a 1:1 peptide ratio be presented to the membrane in order to gain synergistic antibiotic effects and α-helical structuring, perhaps these two-peptide bacteriocins also self-assemble into a functional aggregate prior to forming a complex with membrane lipids. It appears that the elements of similarity in the above examples of synergistic pore-forming antimicrobial peptides suggest a common mechanism of action that may be conserved across the family of α-helical pore forming antimicrobial peptides. Unresolved issues such as specificity in membrane recognition, the mechanism of secondary structural transitioning, and characterization of the structure of the pore-forming complex are important issues currently under investigation.

Duality in peptide antibiotic mechanism

Inhibition of cell wall biosynthesis

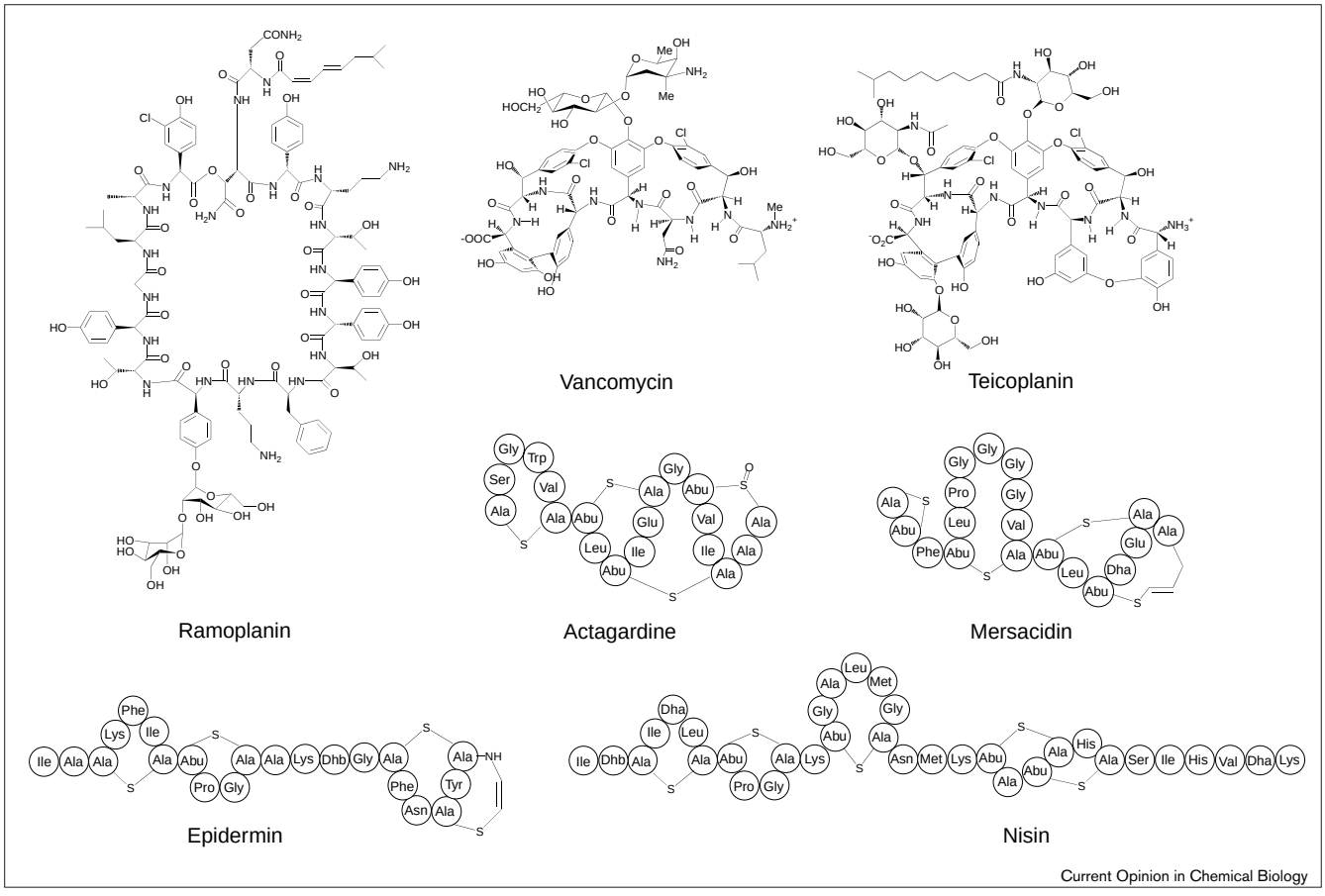
Bacteria have responded to evolutionary and environmental selective pressures by uniquely and elegantly assimilating multiple antimicrobial functions into an individual peptide antibiotic molecule. For example, the glycopeptide antibiotic vancomycin (Figure 3) is a late-stage cell wall biosynthesis inhibitor that acts by blocking formation of cross-links between adjacent peptidoglycan strands [45•]. The vancomycin heptapeptide backbone forms complementary hydrogen bonds with the D-Ala–D-Ala terminus of Lipid II, the cell-surface-anchored peptidoglycan monomer. Once sequestered, Lipid II is physically occluded from utilization as a substrate by cell wall transglycosylase and transpeptidase cross-linking enzymes. Improper cross-linking results in a mechanically weakened cell wall that is susceptible to lysis due to osmotic pressure changes.

Kahne and co-workers [46••] recently discovered a novel carbohydrate-mediated secondary antibiotic mechanism for vancomycin, which does not involve binding of D-Ala–D-Ala. The authors chemically dissected away the vancomycin carboxy-terminal leucyl residue, nullifying D-Ala–D-Ala binding, and found that this derivative and two related hydrophobic *N*-alkylated analogues retained significant antimicrobial activity as inhibitors of peptidoglycan transglycosylation. Further truncation of vancomycin revealed that a C-glycoside containing the unique D-glucose and L-4-*epi*-vancosamine β-linked disaccharide was the primary determinant for the observed transglycosylase antibiotic activity. Their findings suggest that vancomycin acts by two complementary inhibitory modes of action: sequestration of Lipid II and direct interaction with cell wall transglycosylases. Similarly, the carbohydrate-rich glycolipid antibiotic moenomycin A also has been found to exhibit a vancomycin-like dual inhibition of transpeptidation and transglycosylation. In addition to its known inhibitory effect on the penicillin-binding protein (PBP) transglycosylases PBP1a and PBP2a, Graves-Woodward and Pratt [47] have recently determined that moenomycin A inhibits the *Staphylococcus aureus* D-,D-transpeptidase sPBP2a.

Stimulation of host-defense mechanisms

In addition to disruption of membrane integrity, a growing number of antimicrobial peptides possess dual activities in host defense and immunomodulation. Defensins are membrane-active antimicrobial peptides stored in the granules of circulating neutrophils, which are delivered to phagocytotic vacuoles during ingestion of microorganisms [14•]. Defensins not only target cell membrane integrity, but also modulate intracellular signaling events, which lead to stimulation of host-defense leucocyte maturation and anti-infective activities [48,49]. Similarly, dermaseptin, a 34-amino-acid cationic amphibian antimicrobial peptide, forms membrane pores and modulates host-defense by stimulating both the production of reactive oxygen intermediates and exocytosis, two polymorphonuclear leucocyte antibacterial defense mechanisms [16•,50]. Cinnamycin, a 19-amino acid type-B lantibiotic [51•] secreted by *Streptoverticillium griseoverticillatum*, also has antibiotic and immunopotentiating properties. The pore forming activity of cinnamycin observed in phosphatidylethanolamine (PE)-containing membranes arises from the formation of stoichiometric complexes with PE. The immunomodulating properties arise from inhibition of phospholipase A2 activity, which interferes with prostaglandin and leukotriene biosynthesis [52]. Lastly, the antimicrobial peptide granulysin is produced in the granules of cytotoxic T lymphocytes. Granulysin efficiently forms lytic pores in both microbes and tumor cells

Figure 3



Chemical structures of diverse peptide antibiotics that exhibit degeneracy in molecular recognition of a common target – the bacterial peptidoglycan monomer.

and possess a secondary killing function as an inducer of apoptosis in Jurkat cells [53–55].

The highly post-translationally modified type-A lantibiotics nisin, subtilin, Pep5 and epidermin [51•] possess novel secondary modes of action in addition to their well-established lytic activity [51•]. Nisin and subtilin inhibit spore outgrowth in *Bacillus cereus* [56–58]. This antimicrobial activity is mediated by a conserved didehydroalanine residue in position five which is believed to provide a reactive group for interaction with spore-associated factors. Lastly, nisin and Pep5 cause the release of cell wall degrading amidases away from their intrinsic teichoic and teichuronic acid inhibitors, causing autolysis [59].

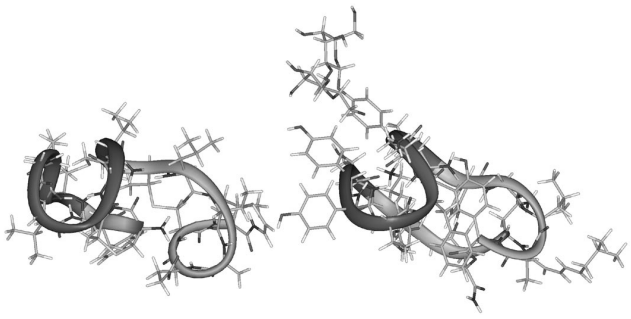
Lipid I and II binding

Conversely, a small but increasing number of structurally dissimilar antibiotics have been shown to interact with the same target molecule. Brotz and co-workers [60•,61] have recently discovered that the lantibiotics nisin and epidermin — but not Pep5 or epilandin — form a complex with the cell wall peptidoglycan monomer, Lipid II, as a cell surface docking molecule [60•]. Nisin and epidermin share a homologous amino terminus, not found in Pep5 or epilandin, which probably is the Lipid II binding site. In addition to nisin and epidermin, Lipid intermediate complexation has been observed for glycopeptides (vancomycin, teicoplanin) and the type-B lantibiotic mersacidin [61]. Although completely unrelated in primary sequence, we have discovered that the lipoglycodepsipeptide ramoplanin exhibits striking peptide backbone homology [62] to mersacidin ([63]; Figure 4) and strongly suggests that the mechanism of ramoplanin inhibition of the peptidoglycan biosynthesis enzyme MurG involves the formation of a complex with Lipid I (Figure 4) (D McCafferty, unpublished data).

Conclusions

Synergy and duality appear to be important evolutionary survival mechanisms that are cleverly reflected in peptide antibiotic design. Thiopeptide and streptogramin-producing organisms have each shrewdly adapted to modulate the function of the bacterial ribosome with small antibiotics. Similarly, organisms that produce membrane-active antimicrobial peptides have elegantly manipulated peptide

Figure 4



Comparison of the three-dimensional NMR structures of the antibiotics Mersacidin and Ramoplanin reveals their highly homologous peptide backbone architecture. **(a)** Mersacidin acts by tightly binding to the peptidoglycan transglycosylase substrate Lipid II. **(b)** Though they have completely different primary sequences, the same structural homology is found in Ramoplanin, strongly suggesting that Ramoplanin acts by binding to the MurG substrate, the structurally related Lipid I.

structure for the purpose of forming pores upon contact with membranes or for traversing these membranes in search of intracellular targets. Likewise, combining dual targeting functions into single antibiotics doubly ensures their antimicrobial success. In an age of widespread antibiotic resistance, multiple antibiotic combinations are often required to overcome infectious disease. Implementing synergy and duality principles into the design of next generation therapeutics is a logical extension of nature's acumen.

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- • of special interest
- •• of outstanding interest

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In this recent examination of thiostrepton mechanism, the authors show that the antibiotic does not interfere with initial binding of elongation factor G (EF-G) or with single round GTPase activity. Instead, antibiotic binding interferes with EF-G function in subsequent steps, namely release of inorganic phosphate after GTP hydrolysis, tRNA translocation, and the dissociation of the factor from the ribosome, thus inhibiting turnover of the ribosomal machinery. The authors also show that thiostrepton interferes with EF-G footprints in the α-sarcin stem loop of the rRNA. It was therefore concluded that thiostrepton binding inhibits a structural transition of the 1067 region of the 23S rRNA that is important for the functions of EF-G after GTP hydrolysis.

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