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Optimizing Antimicrobial Host Defense Peptides

Antimicrobial host defense peptides constitute a major component of innate immune systems. Expectations are high to develop them into a novel class of anti-infective agents. In this issue of *Chemistry & Biology*, Hilpert et al. [1] describe new design and peptide synthesis strategies for systematically investigating such concepts.

Antimicrobial host defense peptides (HDPs) are nature's most abundant antibiotics [2]. Virtually every life form produces an array of peptides which help to create local barriers for competing microbes. Throughout the evolution of higher organisms, microbes were present and there was a vital need to control them; in this scenario, HDPs apparently constitute a useful instrumentarium that was worth conserving and developing [3]. Considering the current dilemma of ever-increasing antibiotic resistance, which makes antibiotics rather short-lived drugs, it remains enigmatic why, after millions of years of application, HDPs are still effective [4].

HDPs are genetically encoded and derive from precursor peptides through proteolytic activation. Close to 1000 naturally occurring HDPs have been described to date from bacteria, fungi, and plants to invertebrates, amphibians, and mammals (http://www.bbcm.univ.trieste.it/~tossi/amsdb.html). There is enormous structural diversity among HDPs, and the only conserved features are (1) small size (10–50 amino acids), (2) an overall positive charge, and (3) amphiphilic properties. Various subgroups have been defined, such as peptides containing disulphide bridges and β sheet or helical segments (e.g., human α- and β-defensins, plectasin, protegrins). These peptides have defined three-dimensional structures in solution and may contain conserved structural motifs [5]. Other classes of HDPs comprise linear α-helical peptides (e.g., cecropins, magainins) and extended peptides (e.g., indolicidin) rich in certain amino acids such as glycine, arginine, proline, and tryptophan [2, 6]. These groups of peptides are highly flexible and they only adopt amphipathic features in the presence of membranes and membrane-mimicking solvents [2, 6].

HDPs Are Multifunctional Antibiotics

As indicated by the descriptive term "antimicrobial peptides," these agents have the widest possible spectrum of activity. They kill gram-negative and gram-positive bacteria as well as fungi and parasites, and they are also able to antagonize viruses. Such a broad activity excludes the existence of a defined molecular target, and it is assumed that HDPs impair the integrity of microbial membranes and cell envelopes (reviewed in [2, 6, 7]). Indeed, the cationic, amphiphilic design of HDPs, i.e., the clustering of charged and hydrophobic side chains in defined surface areas of the molecules, appears to be optimal for such activities. The positive charge ensures accumulation on microbial cell surfaces, which contain acidic polymers such as lipopolysaccharide (LPS) and wall teichoic acids (WTA); it also promotes subsequent interaction with cytoplasmic membranes that, unlike cellular membranes of higher organisms, also have a negative surface charge. This feature provides some target cell specificity and is obviously relevant for the activity of HDPs in vivo, since a major counterstrategy of microbes to reduce their susceptibility to the peptides is based on the reduction of negative charges in the cell envelope [4].

The impact of HDPs on the physical integrity of membranes has been studied intensively on model membranes, and various models exist to describe such activities [2, 6, 7]. However, the actual killing of microbes may require activities of HDPs beyond gross membrane permeabilization. The amphiphilic cationic design enables a maximum of interactions in biological systems, and there are reports on direct effects of cationic peptides on bacterial respiratory chains [8], on cell wall lytic enzymes [9], and on intracellular targets (reviewed in [2, 6]), particularly for some flexible peptides which may pass through the membrane without causing much damage. HDP interference with viral infections may also be mediated through interactions with viral receptors [10, 11]. Full characterization of the activities of these peptides beyond membrane disruption is an area in need of much more attention in future research.

With HDPs, nature has apparently designed "dirty" drugs that simultaneously disturb various biological functions with low potency rather than antibiotics which effectively block one specific target [4]. Since HDPs have been effective for billions of years, the question may be asked, "Could such a concept provide antibiotics with a half-life of more than just a decade, and therefore be considered for future antibiotic drug development?"

HDPs Modulate Innate Immunity

Cationic amphiphilic peptides not only have direct antibiotic effects, but there is increasing evidence that they are also potent modulators of innate immunity [12]. A whole range of immunostimulatory activities is currently being investigated for various HDPs, including attraction of immune cells (classical chemokine activity), modulation of chemokine expression, induction of immune cell differentiation, angiogenesis, and wound healing [13]. The ability of HDPs to bind to microbial surface molecules such as LPS and lipoteichoic acids (LTA), mentioned above in context with the antibiotic activity, adds yet another attractive feature. LPS and LTA strongly upregulate proinflammatory host responses, and the overstimulation of these immune responses frequently leads to fatal septic shock. Thus, peptide binding to these microbial surface molecules interferes with their signaling capacity in inflammation, which

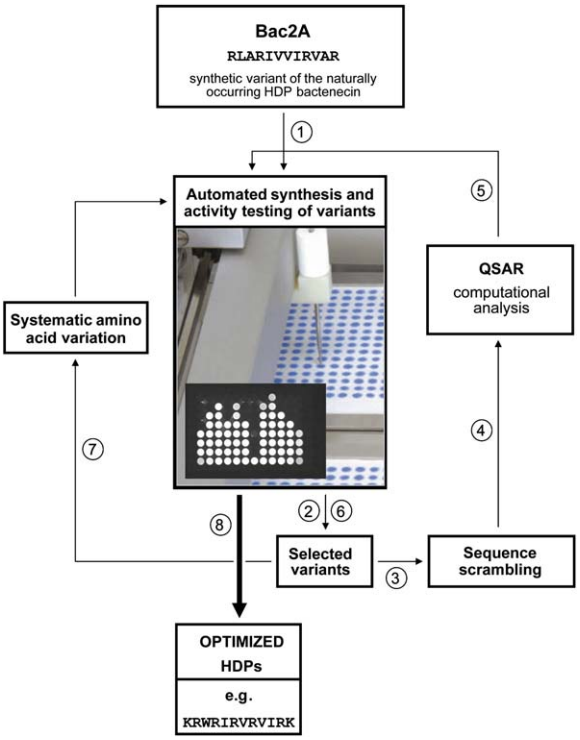

Figure 1. Flow Chart of the Optimization Strategy for Antimicrobial Host Defense Peptides

The sequences of the starting peptide Bac2A and of one of the best-optimized peptides are given using the one-letter code of amino acids. The image shows the robot-assisted spot synthesis of peptides and subsequent activity testing in microtiter plates by means of a luciferase-expressing *P. aeruginosa* strain (inset); loss of light production indicates antimicrobial activity. Image provided by R.E.W. Hancock.

could be beneficial regarding the outcome of severe systemic infections.

HDPs and Drug Development

The prospect of combining all these activities, the direct antibiotic effect, the immunostimulatory effects that improve clearance of microbial loads, and the capacity to neutralize potentially harmful microbial compounds, makes HDPs most attractive for the development of a truly novel anti-infective strategy [14, 15]. However, the road from a promising natural compound to a pharmacologically active anti-infective drug is long, and HDPs so far have had only limited success. Generally, peptides are not amenable to extensive chemical modification, a feature that is considered important in drug development. Also, chemical synthesis of peptides is comparatively expensive and the cost-of-goods problem needs to be considered in the development of peptide-based drugs.

In this regard, Hilpert et al. [1] report on a major technological improvement for the systematic investigation of HDPs. The authors describe a new design strategy for enhancing antibiotic activity which became feasible through a new peptide synthesis technology. Spot synthesis on cellulose supports [16] was combined with subsequent liberation of the peptides and robot-assisted testing for antibiotic activity in microtiter plates. This approach enabled the cost effective production of large numbers of peptides with systematic sequence variations.

The design strategy is based on a synthetic peptide, Bac2A (Figure 1), a derivative of the naturally occurring bovine host defense peptide bactenecin that was identified in previous optimization work with conventional methodology [17]. The new strategy, outlined in Figure 1, included scrambling the sequence of the 12 amino acid peptide, in which peptide size, charge, and hydrophobicity were kept constant. This process identified an essential, short hydrophobic stretch of amino acids that needs to be placed between two positively charged side chains, but may be located anywhere in the sequence. Computational quantitative structure-activity relationship (QSAR) analysis yielded further activity predictions which were experimentally validated. Based on the structure of the best peptide obtained at this stage, another round of optimization was performed using a complete amino acid substitution experiment. Overall, HDPs with a 5- to 10-fold increase in potency, as compared to the Bac2A peptide, were obtained. The minimal inhibitory concentration (MIC) values in the low microgram per milliliter range of concentrations against a broad spectrum of pathogens, including gram-positive, gram-negative bacteria, and a fungal strain, are clinically meaningful and warrant further studies.

In their paper, Hancock and coworkers [1] addressed the direct antibiotic effect only. However, to make use of the full anti-infective capacity of HDPs, it will be necessary to develop and include further automated assays in the optimization process to assess the induction of chemokine synthesis, induction of immune cell differentiation, and wound healing. Technologically, this should be feasible and we may see some of the most exciting concepts in HDP research being systematically analyzed in the near future.

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