BIOCIDES TODAY

Cationic peptide antibiotics: A diversified portfolio for the future

ROBERT E.W. HANCOCK*
ALEKSANDER PATRZYKAT

Department of Microbiology and Immunology,
University of British Columbia
Vancouver, B.C., Canada V6T 1Z3

* E-mail: bob@cmdr.ubc.ca
Fax: +604-822 6041
Tel: +604-822 2682

Over the past decade levels of bacterial resistance to antibiotics have risen dramatically and "superbugs" resistant to most or all available agents have appeared in the clinic. Thus there is a growing need to discover and introduce new drugs. One potential source of novel antibiotics is the cationic antimicrobial peptides, which have been isolated from most living entities as components of their non-specific defenses against infectious organisms (1,2).

Based on these natural templates, scores of structurally diverse antimicrobial cationic peptides have been designed, manufactured both chemically and biologically, and tested for activity against specific pathogens. Although only a few such peptide antibiotics have entered clinical trials to date (3), their diverse portfolio of structures, activity spectra, and modes of action, should provide an invaluable resource to the pharmaceutical chemist.

BIOLOGICAL DIVERSITY

Cationic peptides in nature can be synthesized by multienzyme complexes (gramicidins, polymyxins), or on the ribosome, with or without post-translational modifications (4-6). While the former have furnished antibiotics that are used in current medical practice (polymyxin B, gramicidin S), the latter provide extraordinary opportunity for peptide variation by mutation.

Four structural classes of peptides are widely recognized, including β-sheet peptides stabilized by two to three disulphide bridges, peptides that fold into amphipathic α-helices upon contact with bacterial membranes, extended structure peptides (formed upon membrane contact) that often contain a predominance of one or two amino acids (W, H, or P), and loop peptides. However, there are many variants amongst these basic classes with at least eight sub-classes of β-sheet peptides having been described in plants alone (7). Two main peptide folds have been recognized: amphipathic structures comprising a hydrophilic, positively charged face and a hydrophobic face, and a cationic double wing structure with two pockets of positive charge bracketing a hydrophobic core. Cationic peptides are found in all species of life from bacteria, fungi, insects, crustaceans, amphibians, fish, and birds to mammals, including humans. The fat bodies of insects, the granules of animal leukocytes, and mucosal surfaces exposed to environmental insults are among the most prolific sources of antimicrobial peptides.

Microbiocidal peptides have been reported to successfully kill Gram-negative and Gram-positive bacteria, fungi, enveloped viruses and even cancer cells *in vitro*, but these activities can come at the cost of toxicity to healthy host cells. In such cases, rational modifications of existing peptides and customized delivery methods can reduce peptide toxicity (8,9), as well as enhance the desired activities (10), and increase peptide stability (11). Along with their antimicrobial activities, peptides have been described as agents in wound healing (12-14), as chemoattractants (15-17), and have been reported to bind LPS and consequently provide protection against endotoxic shock (18). The so called "enhancer activity", manifesting as synergy effect with classical antibiotics has also been described.

With the multitude of cationic peptide sources, structures, and spectra of activity come a number of complex and controversial structure-function theories attempting to describe and explain peptide modes of action. Generally speaking, it has been assumed that peptides act

by disrupting the integrity of the bacterial cytoplasmic membrane (although some authors suggest that peptides lyse cells, there is very little evidence for this). Conversely, it has been proposed that leakiness of membranes arises as the peptides traverse the bacterial cytoplasmic membrane and that there are internal targets of peptide action, such as disruption of macromolecular synthesis (19,20). While the mode of action of cationic peptides is not well understood, it is generally agreed that peptides need to interact with cell membranes as part of their action against microbes. This has been the assumption behind many of the rational modifications to the existing peptide structures.

CHEMICAL DIVERSITY

Remarkably only three structural aspects are common to most cationic peptide antimicrobials: they are cationic, with three or more lysines or arginines; they are small, being generally 6-40 amino acids in length; and they tend to contain at least 50% hydrophobic amino acids and their hydrophobic and hydrophilic residues are separated in the folded structure. The latter characteristic supports the contention that interactions with the amphipathic membranes of microbes are critical for peptide action on microbes.

Primary sequence modifications of natural peptides are commonly employed to increase the overall charge or amphipathicity of the peptides, improve their predicted folding patterns, or facilitate production. Some of the most successful alterations include amidation of the C-terminus, and amino acid replacements, insertions or deletions (21,22). We would contend that such approaches will be more successful if changes are introduced based on rational consideration of the 3-dimensional folded structure of the peptides. While the rational approach has shown some success, the very effective peptide CEME was produced by empirically combining the N-terminus of cecropin and the C-terminus of melittin (23). In addition to rationally exploiting the structural patterns among existing cationic peptides, great potential exists for the employment of random techniques such as random combinatorial peptide libraries (24,25) or mutagenesis of DNA sequences encoding such peptides. In this case the number of prospective variants is enormous since there are more than 10^{26} possible 20 residue peptides when only the natural amino acids are considered.

CLINICAL POTENTIAL

Only a few peptides have entered clinical trials, with mixed success - for more information the reader can visit the following web sites *www.intrabiotics.com*, *www.xoma.com*, *www.magainin.com/home.htm*, *www.mbiotech.com*. The research investment required to bring more peptide antibiotics to the clinic will likely remain substantial in the foreseeable future, since any novel class of antibiotics will inevitably raise unique questions. However, the incontestable need for new ways to manage infections, and the proven importance of peptides in innate immunity, should render the investment worthwhile for human and animal medicine. In addition, the overall future potential of cationic peptide antimicrobials far exceeds their limited utilization in conventional pharmaceutical preparations. Several attempts are underway to integrate peptide genes into the genomes of agricultural crops and to render plants resistant to infections (3).

While the scope of this overview does not permit us to exhaust these arguments, the authors believe that most of the existing concerns regarding cationic peptide antibiotics can be addressed by the extensive pool of peptide structural motifs and activities available to the researcher.

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