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Cyclic lipodepsipeptides: time for a concerted action to unlock their application potential?

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“Apparently, the CLP blueprint can produce multifaceted biological agents, and much fundamental insight remains to be gained in the correlation between CLP sequence, structure and biological activity.”

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The range of antimicrobial peptides (AMPs) is very broad and covers a large and diverse group of molecules produced across different kingdoms of life. In a sense, all peptides that possess any activity against any microorganism can be classified as an AMP. Typically however, the AMP label is associated with oligopeptide chains of moderate length featuring two or more positively charged residues. Therefore, the AMP label is often considered synonymous to ‘cationic antimicrobial peptides’, although anionic AMPs also exist, such as maximin H5 or dermcidin. AMPs can be linear or cyclic, the latter typically involving disulfide bonds or the polycyclic thioether amino acid lantionine [1,2]. Quite characteristically, most AMPs only adopt a specific structure or more rigid conformation upon interaction with a membrane, while being flexible and unstructured in solution.

Cyclic lipopeptides (CLPs) can be considered as a subdivision of the AMPs, produced by nonribosomal peptide synthetases in various bacterial species such as *Pseudomonas*, *Bacillus* and *Streptomyces* spp. [3]. CLPs consist of a fatty acid tail linked to a short-to-medium-sized oligopeptide (up to ~20 amino acids), which takes part in a macrocyclic ring structure. The ring is closed by means of an amide or – in the majority of cases – an ester bond. The latter is also referred to as a depsi bond, and such CLPs are thus also called cyclic lipodepsipeptides. Unlike typical AMPs, most CLPs do not carry positive charges, being either uncharged (no ionizable functional groups) or negatively charged. Another feature is that most CLPs – at least for the portion involved in the macrocycle – already adopt a well-defined conformation in solution.

CLP-producing bacteria are found in numerous environments such as plants, soil, organic matter, water and even mammals. The large diversity in bacterial genera and strains that are able to produce CLPs, suggests that these compounds fulfill important functions for their producers [4]. Variations in the fatty acid moiety or in the number, type and configuration of the amino acids create an equally impressive diversity in CLP structures. Consequently, there is no single ‘consensus’ CLP structure but rather a common molecular blueprint. For example, in *Pseudomonas* spp., CLPs such as the bananamides, viscosins, orfamides, amphisins, xantholysins, etc., all appear to be similar isoforms with different peptide lengths, macrocycle sizes, and with some diversity in sequence and stereochemistry. There appears to be a degree of adaptability of the producing organisms in terms of CLP structure relative to environment, growth circumstances, etc.

CLP research

Reported antimicrobial activities for CLPs typically include antibacterial, antifungal and antiviral effects. Over the past few years, this has diversified to include insecticidal and anticarcinogenic activities. Apparently, the CLP blueprint can produce multifaceted biological agents, and much fundamental insight remains to be gained in the correlation between CLP sequence, structure and biological activity. Unfortunately, both the selectivity in biological effects investigated and the diversity in assay procedures prohibit any such analysis to be made (GEUDENS N ET

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AL., UNPUBLISHED DATA). In our opinion, this seriously impedes the further development of CLPs as economically relevant agents in biomedical research. Moreover, we expect the situation to grow worse considering that the rate of discovery of novel metabolites is accelerating through the introduction of high-throughput approaches, such as mass-spectrometry-based molecular network indexing. The latter already allowed the discovery of the bananamides as a novel class of CLPs, and more are expected to emerge as these high-throughput techniques become mainstream [5]. The bottleneck is thus moving toward the biological evaluation.

Like for other metabolite classes, the scientific community has already invested in the development of dedicated databases for CLPs. For instance, the NORINE database catalogs CLP primary structures and their producing organisms [6]. However, depositing sequence, chemical and structural data are insufficient – it must be annotated with biological effects that are preferably extracted from a well-defined set of biological assays if we are to unlock the potential of CLPs. This will require the definition of a common set of microorganisms, reference strains (e.g., ATCC, NCPPB, BCCM, etc.) and pathogens together with established assaying procedures to characterize isolated CLPs. The latter could actively involve free open screening programs for antimicrobial activity such as performed by the Community for Antimicrobial Drug Discovery [7]. In time, this should lead to a database that can be mined for structure–function and structure–activity relationships, while the availability of uniform testing protocols would allow to immediately relate the functional repertoire of a novel, previously undescribed compound to the existing library.

Investigating CLP mode of action

A central research theme for AMPs – and CLPs are no exception – is elucidating the exact mechanism of their antimicrobial activity. For CLPs, the cellular membrane is clearly involved, although the exact mechanism remains unclear. Multiple modes of action have been described including pore formation, protein clustering, fluidity change, etc. Additionally, there may be not a single mechanism and multiple properties may be relevant. For instance, the CLP daptomycin is a so-called calcium-dependent antibiotic, containing a proposed calcium-binding Asp-X-Asp-Gly motif. In complex with Ca^{2+} , daptomycin forms pore-like oligomers on the bacterial cytoplasmic membrane, leading to leakage of intracellular ions, followed by rapid cell death [8]. Moreover, daptomycin interacts with the negatively charged phosphatidylglycerol. This results in a membrane distortion and a delocalization of essential membrane proteins involved in cell division [9]. Membrane interaction studies often suffer from the use of homogenous model membranes, a far cry from the complex architecture of biological membranes. The recent advances in imaging methods which recently showed that daptomycin perturbs microdomains in bacterial cell membranes with concomitant metabolic interferences, bode well for the further understanding of the mechanism of action of AMPs in general and CLPs in particular in biologically relevant models. Indeed, the working mechanism of other cyclic lipodepsipeptides, involving the cellular membrane as primary target and which do not depend on the presence of Ca^{2+} , is still poorly understood [10].

Oligomerization at the membrane interface appears to be a wide-spread phenomenon that potentially plays a relevant role in working mechanism of CLPs [11–13]. For CLPs of the viscosin group produced by *Pseudomonas* spp., a structural proposal for the organization of a cylindrical self-associated complex was put forward whereby the monomer amphipathicity, which is conserved among the viscosin group, is thought to support the self-assembly [13–15]. In contrast, daptomycin appears to undergo a more ‘disordered’ self-assembly in the form of micelles [12]. Further structural studies concerning the nature of the self-assembled structures will be required to gain understanding in the biological relevance of this phenomenon. The biophysical tools are now being developed to distinguish/investigate these different mechanisms [16,17]. At the same time, recent developments allow efficient and rapid synthesis of CLPs, opening the door to synthetic variants that can be used for detailed structure–activity relationship studies [18,19]. Moreover, peptide synthesis can be used to introduce chemical modifications that improve upon biomedical properties, such as the therapeutic window, reduced haemolyticity, etc. [20,21]. Combined, these various developments will in the coming years lead to a breakthrough in uncovering the relation between the various CLP structural features, the antimicrobial activity and selectivity, and the working mechanism.

Concerted action

In the last decade, there has been a significant increase in our understanding of CLPs and their source bacteria. However, challenges will need to be overcome to ensure that the steady stream of scientific results concerning CLPs is accessible for investigation and is sufficiently correlated such that biomedical applications will manifest over time. This will require work in different domains, such as the screening of the biological diversity on different

levels, including structural, microbiological, genetic and ecological and the study of their biophysical and chemical properties. A lot of progress has been made in recent years, and it is expected that this will continue at an increased rate. The CLP community, which so far has blended as isolated researcher groups in the larger world of AMP research, should seek each other out and come together to realize this exciting potential.

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