Cyanobacteria and Microalgae Antibacterial Peptides

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Cyanobacteria and microalgae are oxygen-producing photosynthetic unicellular organisms encompassing a great diversity of species, which are able to grow under all types of extreme environments and exposed to a wide variety of predators and microbial pathogens. The antibacterial compounds described for these organisms include organic compounds such as alkaloids, fatty acids, indoles, macrolides, phenols, pigments and terpenes, among others, but the peptides have an special pharmacological appeal, due to their broad chemical space, achieved by their dual biosynthetic alternatives in cyanobacteria, the ribosomal synthesis, or a polypeptide assembly through the non-ribosomal peptide synthases. This diversity ensures a broad range of biological properties with a large pharmacological potential.

Cyanobacteria,Microalgae,Antibacterial Peptides,

1. Introduction

The advent of the antimicrobial era are, together with sanitation and increasing access to safe drinking water, among the greatest milestones in public health [1]. Antibiotic therapy affords the control of many infectious diseases, otherwise highly lethal. In addition, it pushes forward the boundaries of many other medical treatments, such as immunosuppressive treatments or successful surgical procedures.

The notion of total elimination of infectious diseases by antibiotic therapies soon turned out to be utopic. Today, the world is facing a deep global antimicrobial resistance (AMR) crisis, with an alarming decrease of effectiveness in antibiotic treatments due to the rising resistance acquired by pathogens [2][3]. The overuse, and often misuse, of antimicrobials in clinics [3], is one of the main reasons of AMR, but not the only one. The induction of antibiotic resistance outside nosocomial settings, strongly associated to the antibiotic use in livestock farming [4], aquaculture [5], and the uncontrolled dumping of antibiotics into the environment [6][7], account for the horizontal transmission of antibiotic-resistance traits out of the nosocomial setting. Furthermore, zoonosis may act as a reservoir for resistant organisms. Altogether, the term “One Health” was coined as a common umbrella to encompass all the resistomes, regardless of their biological source, as responsible for induction of resistance [8][9].

This serious situation is worsened by the deficient pipeline for the development of new antibiotic leads, due to the poor return of investments obtained [10][11]. The magnitude of AMR was recognized by the United Nations General Assembly in 2016, that fostered promising initiatives, as the AMR action fund, a multipartner consortium led by the World Health Organization (WHO) expected to put on the market 2–4 new antibiotics by 2020

(https://www.amractionfund.com/). Yet, the end of this crisis will not be achieved in a short time range [12], so immediate solutions must resort to drug repurposing [13][14], or combination therapies, with a simultaneous multitarget attack to the pathogen [15][16]. Thus, the development of new approaches for anti-infectious diseases, such as bacteriophages, enzybiotics, the focus on virulence factors as targets, or the potentiality of CRiSPR-Cas13, is mandatory and urgent [17][18][19][20][21], not only because of the current and alarming situation, but also because of the feasibility to fight emerging ongoing threats, as the COVID-19 pandemics, concerning bacterial co-infections [22].

Among these forefront candidates on trial, are the antimicrobial peptides (AMPs) (for recent reviews, see [23][24][25][26][27][28][29]), which are ancient chemical weapons in the biological welfare. In unicellular organisms, AMPs help the producer cells to strive against competitors sharing the same ecological niche. In pluricellular organisms, they play a defensive role against invading pathogens. The success of AMPs is endorsed by their ubiquitous presence throughout evolution, crossing taxonomical kingdoms [25], even in those organism endowed with a robust and sophisticated antigen-specific immunity. In pluricellular organisms, AMPs may play additional roles out of their primeval function as deterrent for infection, such as messengers for communication among immune cells, angiogenesis, wound healing, autoimmunity [30][31], their dual role in inflammation [32][33], or even in sleep, among others [34][35][36][37][38].

Until few years ago, the pharmaceutical industry was scarcely receptive to peptide-mediated therapies, mostly due to the high cost for production and their poor ADME (absorption, distribution, metabolism, and excretion) profile, despite their huge potential to cover an extremely broad chemical space, and their structural and functional tuning. This concern was driven by the peptide liability to degradation by proteinases and peptidases present in biological fluids, their sequestration by the cellular matrix and serum components, problematic transport across the membranes, as well as the difficulty of the exogenously administered AMPs to reach an effective concentration at deep tissue or organ locations. Most of these shortcomings were addressed and properly solved in recent years, leading to an increasing number of peptide drugs approved by the Food and Drug Administration (FDA) [39][40][41][42].

This turn of the tide underlies new strategies to overcome the limitations described above, converting peptides into valuable drug candidates: firstly, the decrease in cost by implementation of more efficient and cheaper strategies of synthesis [43][44][45][46][47] or, alternatively, the development of improved production of recombinant peptides [48][49][50][51]; secondly, the improvement of peptide bioavailability by engineering strategies aimed to prevent proteolytic degradation, either by manipulation of their primary sequence by incorporation of unnatural amino acids [52][53], β and γ amino acid peptides [54][55], enantiomeric peptides [56][57] and peptidomimetics [58][59][60][61], or by acquisition of a more stable conformation that secludes or shields the recognition of the cleavage sequence by peptidases (cyclation [46][62][63] and stapled peptides [64]).

In addition, the implementation of nanotechnological vehiculation of the peptides improve their bioavailability by targeting the peptide at the right anatomical or cellular location, preventing peptide waste and off-target effects, as well as avoiding the proteolytic degradation of the peptide. In addition, vehicle degradation may sustain or control a

gradual delivery of the peptide at the right site [65][66][67][68]. A greater decrease in the number of peptides entering the pipeline for peptide development is achieved by in silico selection of new and improved prediction tools for candidate selection based on an expected higher effectiveness or decreased toxicity [69][70][71][72][73].

Yet, despite the increase of eukaryotic AMPs that entered into the pipeline and reached different phases in clinical trials [26][27][74], none of them are currently implemented as an over-the-counter drug in the market. In fact, all the AMPs in clinical use are from bacterial origin [75][76][77]: colistin, gramicidin, bacitracin, tyrocidine, the two glycopeptides vancomycin and teicoplanin, the lipopeptide daptomycin A), and the lantibiotic nisin extensively applied as a food preservative.

The cost of an AMPs-based anti-infectious therapy is still significantly higher than for classical antibiotics. However, this drawback is blurred in the case of multiresistant bacteria, being AMPs the last resort drug. Although resistance against eukaryotic AMPs can be induced, their frequency is much lower than for classical antibiotics, due to the high loss of fitness associated [78][79][80]. Nevertheless, a serious clinical concern is the resistance against polymyxin, a lipopeptide used as a last clinical alternative for Gram-negative infections with increase not only in its frequency, but also in its spreading into other bacteria [81][82]. On the other side of the balance, the awareness of the importance of host immune reprogramming by AMPs is a more permanent asset of its overall antimicrobial activity, and presumably, less prone to manipulation by bacterial resistance [27][34].

The search for new natural sources of AMPs has also increased; in this context, microalgae and cyanobacteria have enormous potential as a source of molecules with antimicrobial applications with a high probability of finding new potentially more effective molecules. As a background, these organisms are a source of various chemical substances already characterized, such as peptides, proteins, lipids, vitamins, pigments, carbohydrates, terpenoids, polyunsaturated fatty acids, flavonoids, phenolic compounds, and other organic substances with potential uses as biopharmaceuticals [83].

2. Mechanism of Antibacterial Action of Peptides and Compounds of Cyanobacteria and Microalgae

In general, the mechanism of action of cyanobacterial and microalgae peptides against bacterial cells has not yet been established, and further studies are needed to elucidate the biological activity of these antimicrobial peptides [84][85]. For those antibacterial peptides with a clear cationic character, e.g., from microalgae, we may surmise a mechanism of action rather similar to those peptides described in higher eukaryotes; that is, the disruption of the cell membrane after specific insertion into the bacterial cell membrane. Specificity is mostly achieved by the different electrical charge of the external hemilayer of the cell membrane, negative for prokaryotes and lower eukaryotes, zwitterionic in higher eukaryotes. This mechanism has two important consequences; first, the negative electrical charge of the membrane is considered as a pathogen associated molecular pattern, as such, common to many microorganisms, that makes them susceptible to a given peptide. Secondly, as the bactericidal mechanism is based on the stoichiometric interaction of the peptide with the phospholipids of the lipid bilayer, physicochemical characteristics of the peptide, such as charge, size, amphipaticity, and even secondary structure, are more

important than the primary sequence of the peptide. For others, their mechanism of action differs from membrane disruption, with involvement of intracellular targets, and specificity achieved by subtle recognition between the peptide and its target.

This is the case for some cyanopeptides, as the cyclic peptides brunsvicamides B and C from *Tychonema sp*., reported as inhibitors of phosphatase B of *Mycobacterium tuberculosis*, or the cyclic depsipeptide scyptolin A, isolated from *Scytonema hofmanni*, an inhibitor of a serine protease working as a transpeptidase involved in the bacterial cell wall biosynthesis for certain pathogenic bacteria [86].

Antibiofilm activity is an appealing asset for an antibacterial candidate, as infections in clinical devices are frequently associated to biofilm formation and higher resistance against antibiotics. The cyclic peptides portoamides produced by *Phormidium sp*. display this activity against marine bacteria such as *Cobetia marina*, *Halomonas aquamarina* and *Pseudoalteromonas atlantica*, by inhibition of ATPase H^+-transport activity [87]. This activity has a straightforward application as antifouling agents, and their test as antibiofilm compounds for relevant clinical bacteria is pending.

In some cases, structure-activity relationships were obtained with a variable degree of success, either by sequence comparison among similar cyanopeptides from the same or different cyanobacteria, by genetic mutation, or by chemical synthesis. The antibacterial activity of the lipopeptide schizotrin A against *B. subtilis* has been associated to the presence of a proline linked to the 3-amino-2,5,7,8-tetrahydroxy-10-methylundecanoic acid (Aound), and their uptake into the bacterial cell facilitated by the presence of the fatty acid [88][89]. The dipeptide motif formed by a proline residue bound to the amino group of a 2-hydroxy-3 amino-long chain acid residue is shared for other cyanobacterial cyclopeptides, such as scytonemin A from Scytonema sp [86][90]. The presence of this fatty acid was also identified in lyngbyazothrins A–D, and this acyl chain at position C-5 is relevant for the antibacterial activity of the peptide.

The amino acid analyses of the cyanopeptides lyngbyazothrins A–D reveal three unusual amino acids identified as 4-methoxyhomophenylalanine in A and C, homophenylalanine in B and D, and 3-amino-2,5,7,8-tetrahydroxy-10-methylundecanoic acid (Aound) in A–D; moreover, C and D have an additional *N*-acetyl-*N*-methyltyrosine unit and it seems that the acyl residue at C-5 plays an important role in antimicrobial activity. Schizotrin A and pahayokolides A and B have sequence similarity to lyngbyazothrins. Schizotrin A presents a 4-methoxyhomophenylalanine (Htm) residue, similar to lyngbyazothrins A and C, bound to the Pro-Aound-Gln-Gly-Pro sequence, common to all of the lyngbyazothrins. The same sequential motif is also found in pahayokolides A and B but, in contrast to schizotrin A, it is linked to an homophenylalanine. Significant differences were found for the remaining five residues of the cyclic systems among the three classes of peptides: Phe-Val-Ser-DeHThr-Ser in schizotrin A, Phe-Z-Dhb-Ser-E-DhB-Thr in pahayokolides and Pro-allo-Ile-Ser-DeHThr-Thr in lyngbyazothrins. The free hydroxyl group at C-5 of the Aound residue in lyngbyazothrins A and B is substituted by N-acetyl-N-methyltyrosine in C and D instead of the N-butyryl-N- methylalanine residue in schizotrin A. On the other hand, schizotrin A and pahayokolide A contain the aliphatic amino acids alanine and leucine, while lyngbyazothrins C and D include the aromatic amino acid tyrosine; it has been proposed that the nature of these amino acids may also

account for the activity of lyngbyazothrins against the Gram-negative bacterium *E. coli*, absent in schizotrin and pahayokolide [89].

The ribosomal cyclopeptide aeruginazole A isolated from the cyanobacterium *Microcystis sp.* (IL-323), inhibits the growth of *B. subtilis* and *S. aureus*; in its cyclic structure it contains three subsequent glycine residues plus L-Val, L-Phe, thiazole-L-Val, thiazole-D-Leu, D-Tyr and thiazole-l-asp. Similarly, aeruginazole DA1497 isolated from *M. aeruginosa*, is a large cyclopeptide with four thiazole (tzl) moieties, having a cyclo-structure of (-(tzl-phe)-gly-ala-ile-(tzl-ala)-ser-(tzl-val)-pro-gly-val-(tzl-leu)-pro-gly-). It seems that the larger size and the greater number of thiazole groups of these compounds may be associated with their bioactivity. Only DA1497 out of the one of five aeruginazole peptides tested (DA1304, DA1274, DA1338 and DA1372) was active against *S. aureus*, even when minor differential sequential variations occurred among the five peptides [91]. The cyclic lipopeptide Trichormamide C from *Oscillatoria sp.* UIC 10,045 is characterized by the presence of three non-proteinogenic α-amino acid residues (N-methyl-Ile and two 3-hydroxy-Leu) and one β-amino acid, with a key role on its anti-*M. tuberculosis* activity [92].

The antibacterial mechanisms of microalgal peptides have been scarcely reported to date. The few references on the subject refer to extracts or protein hydrolysates, and not to specific peptides. Microalgal extracts from the species *Leptocylindrus danicus* (FE322) and *L. aporus* (FE332) strongly inhibited the biofilm formation by the bacteria *Staphylococcus epidermidis*, but did not show cytotoxicity by standard antibacterial tests [93]. Tejano et al. [94], reported a higher antibacterial activity on Gram-positive than on Gram-negative bacteria for the pepsin hydrolysate and the peptide fractions from *Chlorella sorokiniana*, likely associated to a hindered penetration of the peptide by the outer membrane.

It has been proposed that microalgal compounds with antibacterial activity are released after the loss of algal integrity, or alternatively induced by the presence of bacteria or other pathogens. For other microalgal compounds involved in a defense mechanism against predators and pathogenic bacteria, it appears that the bacterial cell membrane would be the main site of action. There is some evidence of deleterious effects of fatty acids on the bacterial membrane, causing cell leakage, a reduction in nutrient intake and a reduction in cellular respiration. The antibacterial action of fatty acids can also be mediated by the inhibition of the synthesis of bacterial fatty acids; this effect could be bactericidal or bacteriostatic preventing bacterial multiplication. It has also been reported that antibacterial exometabolites released by *T. suecica* inhibited several Vibrio species in vitro causing a rapid decrease in bacterial mobility with cells elongation and vacuolization [95].

Advances in the knowledge of the mechanisms of action underlying the bactericidal activity of peptides from cyanobacteria and microalgae will contribute to the development of these peptides as novel drugs. The role of “omics” techniques in this process, more specially proteomics and peptidomics, will push forward the boundaries for this field [84][85].

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