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Research review paper

Lipopeptides in microbial infection control: Scope and reality for industry

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ABSTRACT

Lipopeptides are compounds that are formed by cyclic or short linear peptides linked with a lipid tail or other lipophilic molecules. Recently, several lipopeptides were characterized, showing surfactant, antimicrobial and cytotoxic activities. The properties of lipopeptides may lead to applications in diverse industrial fields including the pharmaceutical industry as conventional antibiotics; the cosmetic industry for dermatological product development due to surfactant and anti-wrinkle properties; in food production acting as emulsifiers in various foodstuffs; and also in the field of biotechnology as biosurfactants. Some lipopeptides have reached a commercial antibiotic status, such as daptomycin, caspofungin, micafungin, and anidulafungin. This will be the focus of this review. Moreover, the review presented here will focus on the biotechnological utilization of lipopeptides in different fields as well as the functional–structure relation, connecting recent aspects of synthesis and structure diversity.

Contents

1. Introduction

Antimicrobial peptides are multifunctional compounds with multiple utilities (Franco, 2011). Among them, lipopeptides are small molecules that are formed by cyclic or short linear peptides linked with a lipid tail or other lipophilic molecules (Arnusch et al., 2012; Raaijmakers et al., 2010). The first lipopeptide discovered with an antimicrobial function, polymyxin A, was isolated in 1949 from the soil bacterium *Bacillus polymyxa* (Jones, 1949), but the biosynthesis of these molecules has been detected in several bacterial genera, mainly *Bacillus*, *Pseudomonas* and *Streptomyces*, as well as in fungi. Recently, several lipopeptides have been characterized, showing diverse activities like surfactant, antimicrobial and cytotoxic (Raaijmakers et al., 2010). Some of them have even reached a commercial antibiotic status, like daptomycin (Robbel and Marahiel, 2010), caspofungin (Ngai et al., 2011), micafungin (Emiroglu, 2011) and anidulafungin (George and Reboli, 2012).

Currently, lipopeptide properties may lead to applications in diverse areas of industry. In the pharmaceutical industry, lipopeptides have been used when conventional antibiotics were no longer working against resistant bacteria or fungi. In the cosmetic industry, surfactant and anti-wrinkle characteristics of lipopeptides are applied in dermatological products, since lipopeptides present low cytotoxicity against human cells. In food production, lipopeptides are used as emulsifiers in various foodstuffs. Finally, lipopeptides are applied in biotechnology as biosurfactants, giving rise to several industrial and medical applications.

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With this in mind, the function–structure relation of lipopeptides, reporting recent aspects in synthesis and structure diversity, as well as several applications of these antimicrobial molecules in pharmaceutical, cosmetic, food and biotech industries will be discussed here.

2. Lipopeptides' mechanism of action and resistance

Several questions have been raised over lipopetides' mechanisms of action. Data have shown that pore formation in membranes occurs after lipopeptide oligomer binding, some of which are Ca^{2+} dependent multimers (Scott et al., 2007). These pores may cause transmembrane ion influxes, including Na^{+} and K^{+}, which result in membrane disruption and cell death (Mangoni and Shai, 2011; Ostroumova et al., 2010; Scott et al., 2007). In the case of daptomycin, oligomer formation probably occurs before membrane contact. This process seems to be related to ion binding, as after calcium binding the lipopeptide undergoes a conformational modification that leads to oligomerization, even in high concentrations. Daptomycin/calcium complex interacts with the negatively charged membrane phosphatidylglycerol head groups and undertakes a second conformational alteration that induces oligomerization of the membrane, leading to membrane penetration (Muraih et al., 2011).

In addition, some research has showed that lipopeptides can inhibit fungi cell wall formation (Schneider and Sahl, 2010a, b). Echinocardins act by specific and non-competitive inhibition of enzyme β-(1,3)-D-glucan synthase (Yao et al., 2012). This carbohydrate is an essential component for the fungal cell wall to keep its structural integrity. The lack of β-(1,3)- D-glucan leads to cell wall deterioration and consequential cell death (Yao et al., 2012). The relationship between structure and activity has also recently been verified for the echinocardins (Fig. 2F). The authors suggested that chemical modifications in some parts of the molecule, like alterations in non-proteinogenic amino acids or in the fatty acid chain, may increase its activity or modify the specificity (Yao et al., 2012). However, it is not only the membrane that could be the main target of lipopeptides. For example, at lower concentrations, the antifungal lipopeptide from *Bacillus amyloliquefaciens* can cause apoptosis by binding to ATPase on the mitochondrial membrane (Qi et al., 2010).

These modes of action may confer upon lipopeptides' high activity against multidrug-resistant bacteria (Mangoni and Shai, 2011), and the emergence of resistance against lipopeptides is extremely rare (Sader et al., 2011). One example occurs in the bacterial resistance process of lipopeptide daptomycin in *Bacillus subtilis* after artificial selection (Hachmann et al., 2011). Changes in phosphatidylglycerol content in *B. subtilis* mutants explain this resistance, probably due to a decrease in the net negative charge of the membrane which reduces the interaction with daptomycin-Ca^{2+} complex (Hachmann et al., 2011). Otherwise, the emergence of lipopeptide resistance that inhibits peptidoglycan synthesis, like MX-2401, seems to be more unlikely (Rubinchik et al., 2011). MX-2401 binds to undecaprenylphosphate, a carbohydrate carrier involved in several biosynthetic pathways, and this linkage results in several cell wall precursors in biosynthesis inhibition (Rubinchik et al., 2011).

3. Origin and structural diversity of lipopeptides

Lipopeptides with antimicrobial activity have been found in a wide number of microorganisms, and have been purified from the following bacterial genera: *Actinoplanes* (Schneider et al., 2009), *Bacillus* (Velho et al., 2011; Yuan et al., 2011), *Brevibacillus*(Desjardine et al., 2007), *Lyngbya* (Balunas et al., 2010), *Paenibacillus*(Guo et al., 2012; Qian et al., 2012), *Pseudomonas* (de Bruijn and Raaijmakers, 2009; de Bruijn et al., 2008), *Streptomyces* (Alexander et al., 2011; Gu et al., 2011), *Tolypothrix* (Neuhof et al., 2005; Neuhof et al., 2006), and in the fungi *Aspergillus nidulans* (Cortes and Russi, 2011; De Lucca and Walsh, 1999). All of these natural lipopeptide sources provide high levels of structure diversity reflecting several modes of action and targets (Tally et al.,1999). There are variations in length, configuration, number, and composition of lipids and amino acids in the structure.

Lipopeptides are synthesized in microorganisms by nonribosomal peptide synthetases (NRPSs) (Mitchell et al., 2012). These enzymes possess a modular structure formed by multiple catalytic domains that act like a multidomain protein (Mitchell et al., 2012). There are three domains in the NRPSs, each one with a different function that includes adenylation (domain A), condensation (domain C) and thioesterase (TE) (Fig. 1). At the beginning of the synthesis process, amino acids and peptides are adenylated and then covalently linked to the peptidyl carrier protein (PCP). The variation of selected amino acid residues in NRPSs can explain the variation in the lipopeptide amino acid sequence. Following PCP linkage, the NRPS condensation domain catalyzes the peptide bond formation between two amino acids. Ending the reaction, peptide release is performed after thioester hydrolysis (Mitchell et al., 2012). This final step, in most cases, catalyzes the cyclization of mature lipopeptides (Samel et al., 2006). The fatty acid moiety is linked at the peptide N-terminus by the action of several enzymes (Hansen et al., 2007; Kraas et al., 2010, 2012), and this variable mode of synthesis produces several kinds of fatty acid chains in lipopeptides.

In nature, variations in selected amino acids and fatty acids during lipopeptide biosynthesis by microorganisms can yield a vast and different number of molecules. They could present differences in amino acid composition, in structure, being linear (Desjardine et al., 2007) or cyclic (Schneider et al., 2009), and in composition and also fatty acid chain length (Fig. 2). In counterpart, the artificial chemical synthesis of lipopeptides has been performed using solution phase synthesis [3 + 3] (Yao et al., 2012), solid-phase synthesis (Brunsveld et al., 2006) and by the chemoenzymatic approach (Grunewald and Marahiel, 2006). However, solid-phase chemistry produces purified peptides in less time and with superior overall yields (Brunsveld et al., 2006). Modifications in these chemical synthesis methods can be applied to simplify and optimize the lipopeptide structure (Yao et al., 2012) and even by reduction of the peptide length or lipid tail, the artificial lipopeptides show similar activities and mode of action. Makovitzki et al. (2006) show that short artificial lipopeptides, with only 4 amino acid residues and a 16 carbons fatty acid chain, are active against Gram-positive bacteria and fungi. Likewise, it has been demonstrated that modifications in lipid tail length may change lipopeptides' activities and specificities; for example, lipopeptides synthesized with 10 or 12 carbon atoms at the lipid tail seem to be non-hemolytic and active towards both bacteria and fungi, whereas larger lipopeptides, with 14 or 16 carbon atoms, showed enhanced efficiency in fungal control (Malina and Shai, 2005). Further modifications, like the replacement of lipid tail by lipophilic biomolecules, such as vitamin E and cholesterol, increased the activity against fungi and simultaneously reduced the hemolytic activity; Nevertheless, the motives for this are still not known (Arnusch et al., 2012). In addition, the cyclization of chemically synthetized lipopeptides can be aided using NRPS TE excised domains (Grunewald and Marahiel, 2006).

Among linear lipopeptides from natural sources, tauramadine and dragomide E could be cited. Tauramadine (Fig. 2A) is a linear lipopeptide, isolated from *Brevibacillus laterosporus*, with five amino acids and a fatty acid tail with six carbons (Desjardine et al., 2007). Dragomide E (Fig. 2B) is a linear lipopeptide from *Lyngbya majuscule* that shows five amino acids chain-linked in their structure with a five-carbon fatty acid tail (Balunas et al., 2010). Otherwise, cyclic lipopeptides were also widely found. The lipopeptide friulimicin (Fig. 2C), isolated from *Actinoplanes friuliensis*, is formed by a cyclic peptide with 10 amino acid residues and a branched fatty acid side chain with 11 carbons (Schneider et al., 2009). Another pair of cyclic lipopeptides was isolated from the *Paenibacillus* bacterial genus. The first consists of a cyclic lipopeptide with a 15 fatty acid chain and 13 amino acid residues (FA-Orn-Val-Thr-Orn-Ser-Val-Lys-Ser-Ile-Pro-