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Review of surfactin chemical properties and the potential biomedical applications

Review Article

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Abstract: Surfactin, a highly powerful biosurfactant produced by various strains of the genus *Bacillus*, exhibits antibacterial, antiviral, antitumor and hemolytic action. This anionic cyclic lipopeptide is constituted by a heptapeptide interlinked with a β-hydroxy fatty acid. Due to its amhipathic nature surfactin incorporates into the phospholipid bilayer and induces permeabilization and perturbation of target cells. The rising antibiotic resistance as well as a number of remarkable surfactin activities shows that it deserves special interest and is considered as a candidate compound for combating several health related issues. In this review, the current state of knowledge of surfactin properties, biomedical potential and limitations for its application is presented.

Keywords: *Antimicrobial resistance • Biosurfactants • Lipopeptide antibiotics • Membrane • Surfactin • Therapeutic agents*

1. Introduction

All classes of living forms ranging from prokaryotes to humans are able to produce diverse antimicrobial compounds with an amazing variety of structures and mode of actions [1,2]. Owing to important medical properties some of these compounds deserve special interest triggered by the increasing demand for effective antibacterial, antiviral and antitumor agents.

Two categories of compounds have recently been focused on - cationic antimicrobial peptides and lipopeptides. The former are ubiquitous molecules in nature (e.g. alamethicin, mellitin) that play a fundamental role in the immunity of the producers. A number of natural or derived cationic peptides are in various stages of development or even in a certain phase of clinical trials as promising agents that can complement conventional antibiotic therapy [3,4].

Lipopeptides represent a unique class of cyclic peptides with either a net positive (e.g. polymyxin) or net negative charge (e.g. surfactin, daptomycin). These bioactive secondary metabolites exhibit remarkable therapeutic and biotechnological properties. Surfactin, one of the principal representatives of the antimicrobial lipopeptide family, is produced by *B. subtilis* and displays an astonishing array of actions. The history of this surface-active agent dates back to 1968, when a new biologically active compound was observed in the culture broth of a *Bacillus subtilis* strain [5]. Consequently, its structure as a macrolide lipopeptide was elucidated [6]. Although other lipopeptide antibiotics have been discovered since, surfactin is best known for its multifaceted interactions with biological systems that result in a number of physiological and biochemical activities. These characteristics make surfactin a candidate drug for solving a number of global public health issues.

2. Definition of surfactants

Surfactants are defined as substances that absorb to and alter the conditions prevailing at interfaces [7]. These amphipathic molecules partition preferentially at the interface between two phases of different degrees of polarity and hydrogen bonding, such as the oil and water or air and water interface.

Surfactants reduce the free energy of the system by replacing the bulk molecules of higher energy at an interface. The effectiveness of a surfactant is

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determined by its ability to lower the surface tension, which is a measure of the surface free energy per unit area required to bring a molecule from the bulk phase to the surface [8]. Due to the presence of a surfactant, less work is required and the surface tension is reduced. The surface tension correlates with the concentration of the surface-active compound until the critical micelle concentration (CMC) is reached. The CMC is defined as the minimum concentration necessary to initiate micelle formation [9]. In practice, the CMC is the maximum concentration of surfactant monomers in water that is influenced by pH, temperature and ionic strength. Efficient surfactants have a low CMC, i.e. less surfactant is necessary to decrease the surface tension.

Microorganisms, having a large surface-to-volume ratio, produce a variety of surface-active compounds (surfactants) referred to as biosurfactants. These compounds have advantages over their chemical counterparts because they are biodegradable and less toxic, and are effective at extreme temperatures and pH [7,10]. The biodegradability of most anionic lipopeptides stems from the presence of Asp-Gly segments in their peptide moieties, making them prone to chemical reaction and degradation under physiological conditions [11].

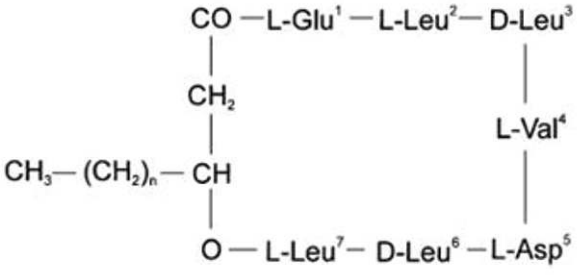
These characteristics make biosurfactants and namely surfactin highly potent agents with diverse commercial applications such as subsurface pollution remediation, the enhancement of the availability of hydrophobic compounds (e.g. oil, polyaromatic hydrocarbons, pesticides), thus increasing their potential for microbial degradation [8]. However, high production costs limit its use in these high-volume applications [12]. On the other hand, the relatively low (μM) effective concentration in biological systems could facilitate the usage in biomedicine.

Surfactin is synthesized by several strains of the genus *Bacillus* during the stationary phase when nutrients in the culture media are limited. Its role in bacterial physiology has been studied intensively using mutants defective in surfactin production. Various functions were impaired in these strains including the development of swarming colonies on the solid media [13,14]. Some other possible roles have also been proposed: an increase of the surface area of hydrophobic water-insoluble growth substrates that results in the higher bioavailability of nutrients, and the influence on the attachment and detachment of bacterial cells to and from substrates [7]. Surfactin also has a function in biofilm formation [15]. It becomes obvious that this type of antibiotic plays a crucial role in the communal development of *B. subtilis* and contributes to its survival in the natural habitat.

3. Structure and physico-chemical properties of surfactin

Surfactin (M.W. 1036 Da), an amphipathic cyclic lipopeptide, is constituted by a heptapeptide (ELLVDLL) with the chiral sequence LLDLLDL interlinked with β-hydroxy fatty acid of the chain lengths 12 to 16 carbon atoms to form a cyclic lactone ring structure (Figure 1). Hydrophobic amino acid residues are located at positions 2, 3, 4, 6 and 7, while the glutamyl and aspartyl residues at position 1 and 5, respectively, introduce two negative charges to the molecule. Several surfactin isoforms usually coexist in the cell as a mixture of several peptidic variants [16,17] with a different aliphatic chain length [18]. In aqueous phase as well as at the water/air interface, surfactin adopts a β-sheet structure with a characteristic horse-saddle conformation, which is probably responsible for its broad spectrum of biological activities [19].

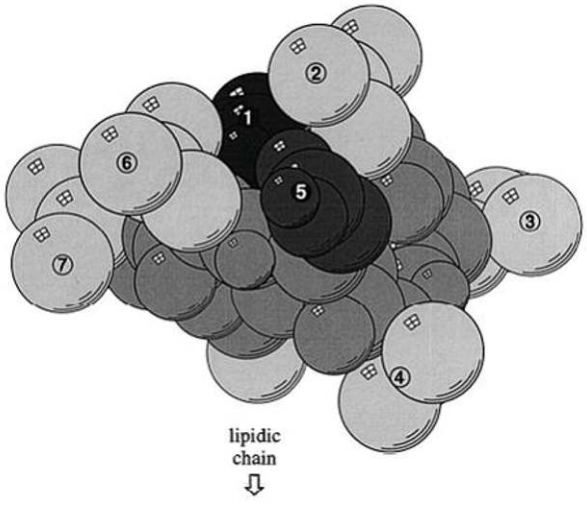
Figure 1. Primary structure of surfactin (n = 9-11).



High resolution ^{1}H NMR combined with molecular imaging techniques determined the three-dimensional structure of surfactin (Figure 2). On one side of the molecule residues 2 and 6 face each other in the vicinity of the acidic Glu-1 and Asp-5 side chains, which define a minor polar domain [20]. On the opposite side, residue 4 faces the connection of the lipidic chain constituting a major hydrophobic domain, which includes the side-chains of residues 3 and 7 to a lesser extent, accounting for its amphiphilic nature and its strong surfactant properties [21]. Below CMC the lipidic chain should extend freely in solution but it strongly participates in hydrophobic interactions in supramolecular structures such as lipid micelles or oligomers at the air/water interface [22].

Surfactin, as one of the most powerful biosurfactants, lowers the surface tension of water from 72 $mN.m^{-1}$ to 27 $mN.m^{-1}$ at a concentration as low as 10 μM, which is far below the critical micelle concentration in water of 23 mg/l and about two orders of magnitude smaller than those of most other detergents. Surfactin forms rod-like micelles with an aggregation number of ~ 170 [23].

Figure 2. The three-dimensional structure of surfactin peptide moiety. Backbone atoms are in grey. Heavy atoms of amino acid residues (1 to 7) are presented. Pale grey - hydrophobic residues 2, 3, 4, 6, 7 and the attachment of the lipidic chain. Acidic residues 1 and 5 are in black and dark grey respectively [22].



4. Surfactin-membrane interaction

Surfactin as a molecule of an amphiphilic nature destabilizes the membrane and disturbs its integrity [24]. A key step for membrane destabilization and leakage is the dimerization of surfactin into the bilayer [25]. The hypothetical mechanisms of surfactin interactions with membrane structures exhibits a complex pattern of effects such as insertion into lipid bilayers, chelating mono- and divalent cations, modification of membrane permeability by channel formation or membrane solubilization by a detergent-like mechanism.

Surfactin penetrates into the membrane through hydrophobic interactions, thus influencing both the hydrocarbon chains order and the membrane thickness. Then, upon this primary collision, the peptide cycle displays conformational changes which further facilitate the interaction process [26]. *In vitro* the incorporation of surfactin into the membrane gives rise to dehydration of the phospholipid polar head groups. Local dehydration and perturbations of lipid packing have been shown to strongly compromise bilayer stability, leading to the disturbance of the membrane barrier properties. These structural fluctuations may well explain the primary mode of the antibiotic action and the other important biological effects of this lipopeptide [25].

The extent of perturbation of the phospholipid bilayer correlates with the concentration of surfactin. Even at low concentrations surfactin penetrates readily into the cell membrane, where it is completely miscible with the phospholipids and forms mixed micelles. At moderate concentrations, the lipopeptide forms domains segregated within the phospholipid bilayer that may contribute to the formation of ion-conducting pores in the membrane [27]. At high concentrations, the detergent effect prevails and it results in the disruption of the membrane. The local surfactin-to-lipid ratio (R_b) within the membrane necessary to start the leakage is $R_b \sim 0.05$ [28]. The permeabilizing activity of surfactin is thus stronger than that of detergents such as Triton.

Membrane penetration by surfactin is facilitated in the presence of cations [26]. The two acidic residues Glu-1 and Asp-5 form a well-suited “claw”, which can easily stabilize a surfactin-Ca^{2+} 1:1 complex via an intramolecular bridge [29]. This effect of Ca^{2+} ions on the surfactin conformation promotes the deeper insertion of the lipopeptide into the membrane [27,30].

Surfactin can also drive mono- and divalent cations through an organic barrier, divalent cations being transported with greater efficiency [31]. The selective affinity can be correlated with the partial neutralization of the two acidic residues at the air/water interface in the presence of Na^+ or K^+, whereas Ca^{2+} induces a complete neutralization [29]. One physiological result of the cation chelation is the inhibition of the cyclic AMP phosphodiesterase activity [32].

Variations both in the peptide and lipid moiety of the surfactin molecule can profoundly modulate the structure-function relationship. Within the large hydrophobic domain, position 4 showed a high contribution since the L-Val4/L-Ile4 substitution induced a 2-fold decrease of the CMC and a substantial gain of the monolayer stability at the air/water interface [20]. With regards to the effect of the acyl chain length, a higher surface activity was observed with a C14 acyl chain while the antiviral and hemolytic actions were stronger when the C15 chain prevailed in surfactin [16]. These actions were amplified when the peptide ring contained a single negative charge and conversely, the replacement of a carboxyl group by a bulky strong negative charge almost completely abolished the surface activity [33].

5. Surfactin biosynthesis

The ability of surfactin synthesis is widely distributed not only among *B. subtilis* but also among *B. pumilus*, *B. licheniformis* and *B. amyloliquefaciens* strains. Similar to other peptide secondary metabolites of bacteria, the synthesis of surfactin is realized nonribosomally. As early as in the 1970s it was discovered that the cyclic peptides gramicidin S and tyrocidine from *Bacillus* spp. were produced in a nucleic acid-independent way through the use of large enzyme complexes similar

to fatty acid synthetases [34]. Subsequently, other peptidic natural products were shown to be assembled by large enzymes, referred to as nonribosomal peptide synthetases (NRPS), which utilize the multiple carrier thiotemplate mechanism. The nonribosomal machinery for peptide synthesis uses these complexes as an assembly line to catalyze stepwise peptide condensation. The substrates are not restricted to the 20 amino acids, since hundreds of building blocks are now known to be integrated and modified by postsynthetic action. Typical of this assembly line is the incorporation of nonproteinogenic amino acids, such as D-isomers, carboxy acids, and N-methylated residues, as well as the incorporation of heterocyclic rings and fatty acids. Glycosylation and oxidative cross-linking are common additional postsynthetic modifications by enzymes that are associated with the NRPS machinery [35].

Studies on the biosynthesis of surfactin began with the work of Kluge et al. [36], who proposed a non-ribosomal mechanism catalyzed by multienzymatic thiotemplates constituting the surfactin synthetase. The scheme of biosynthesis is based on the multiple-carrier concept, implying multiple 4´-phosphopantetheinyl cofactors (ppan) that functions as a swinging arm serving as an acceptor of the growing peptide chain and donor of the peptide to the next thiolester-linked amino acid in the template sequence. All of the seven amino acids constituting the heptapeptide are activated through an ATP-dependent adenylation and subsequently, they are tethered via a carboxythioester bond to the distinct enzymatic modules [37,38].

The surfactin synthetase complex consists of four enzymatic subunits. Three of them are enzymes SrfA (E_{1A}, 402 kDa), SrfB (E_{1B}, 401 kDa) and SrfC (E_2, 144 kDa), which form seven modules that comprise 24 catalytic domains. The last protein of the complex is SrfD (E_3, 40 kDa) that plays an important role in the surfactin initiation reaction [39]. Each module is responsible for the specific incorporation of one dedicated substrate to the growing heptapeptide chain [22].

The first step of biosynthesis is the recognition and activation of a dedicated substrate by the adenylation domain (A domain, about 550 amino acids) [40]. By analogy to aminoacyl-tRNA synthetase, the A domain catalyzes the activation of a substrate as aminoacyladenylate through the Mg^{2+}-dependent hydrolysis of ATP and the release of pyrophosphate [41]. In the next step, the aminoacyladenylate intermediate is transferred to the free thiol group of the ppan cofactor, which is tethered to the thiolation domain (T domain, or peptidyl carrier protein, about 80 amino acids) located downstream of the A domain [42]. The intermediates, tethered to the flexible ppan cofactor, can be transferred to other domains for subsequent catalytic reactions. Peptide bond formation between two adjacent substrates is catalyzed by the condensation domain (C domain, about 450 amino acids), which is located between the A and T domains of subsequent modules [43]. The C domain catalyzes the nucleophilic attack of the amino acid bound to the downstream T domain with its free α-amino group on the activated thioester of the upstream T-domain-bound intermediate [44].

SrfD plays an important role in the surfactin initiation reaction. The N-terminal L-Glu-activating module of surfactin synthetase is able to bind β-hydroxy fatty acid substrate and to connect it to the start amino acid L-glutamate, thus initiating surfactin biosynthesis. SrfD protein mediates the transfer of the fatty acid substrate to the Glu-module and stimulates β-hydroxyacyl-glutamate formation. Surfactin formation can be achieved by the three SrfA-C subunits alone however; biosynthesis of surfactin is stimulated by SrfD at the level of the initiation process [39]. This acyltransferase enzyme also possesses an external thioesterase activity (SrfTE-II), which generally may be involved in proofreading functions to eliminate incorrect charging of the ppan cofactor at the reaction centers of peptide synthetases [45].

The subunits SrfA and SrfB catalyze the elongation of the initiation product through a series of thioester bond cleavages and simultaneous transpeptidation reactions. Finally, the subunit SrfC catalyzes the condensation of the last amino acid residue and the release of the resulting lipoheptapeptidyl intermediate from the biosynthetic complex [22]. This reaction is usually accomplished by a thioesterase domain (TE-I, about 280 amino acids) fused to the SrfC. The peptide can be released either by hydrolysis as a linear acid or by an intramolecular reaction with an internal nucleophile to give a cyclic peptide. The intramolecular lactonization to the initiating β-OH fatty acyl moiety possibly involves a second thioesterase, i.e. the above mentioned SrfTE-II [46,47].

Besides A, T, and C domains, there are other optional domains such as the epimerization domain E (about 450 amino acids) that catalyzes the racemization of the T-domain-bound amino acid. The adjacent C domain incorporates only the D-amino acid into the growing peptide chain [48]. In the surfactin synthetase, two epimerization domains in modules 3 and 6 are responsible for the racemization of T-domain-bound L-Leu. The combination of L- and D-amino acids gives the peptide a unique conformation that is important for the specific interaction with its cellular target.

The principle of surfactin excretion from the producer remains unclear. As no active transporter has been

identified so far, it is widely assumed that surfactin passively diffuses across the cytoplasmic membrane [49].

5.1. Biosynthetic genes and the regulation of surfactin biosynthesis

The assembly of the surfactin synthetase enzymatic complex is reflected in the chromosomal organization of its genes. Bacterial peptide synthetases have two common features that appear to be strictly conserved: the enzyme subunits are co-regulated at both transcriptional and translational level and the organization of the different enzymatic domains fulfills the "colinearity rule" according to which the order of the domains parallels their function hierarchy. By contrast, it is not true for surfactin synthetase. Specific protein-protein interaction rather than the ordered position and the progressive expression of the enzyme domains/subunits dictates the way in which peptide synthetases correctly assemble [50].

The surfactin synthetase complex is coded by the inducible operon named *srfA* (25 kb), which is also responsible for sporulation and competence development [51]. It contains four modular open reading frames ORF1 (srfA-A), ORF2 (srfA-B), ORF3 (srfA-C) and ORF4 (srfA-D) encoding the four respective enzymes. The two nucleotide regions for the L-Leucine epimerases are located at the 3´ portion of the srfA-A and srfA-B genes. At the end of srfA-C gene, one region codes for an enzyme homologous to fatty acid thioesterases type I. Another thioesterase region, which corresponds to the fourth reading frame srfA-D, encodes a protein bearing more sequence homologies with the type II thioesterases of mammalian cells [22].

The second gene essential to the production of surfactin is *sfp* mapped at 4 kb downstream of the *srfA* operon [52]. The encoded enzyme (224 amino acids) belongs to the superfamily of 4´-phosphopantetheinases that function as primers of the non-ribosomal peptide synthesis. Another function of the Sfp enzyme is the conversion of the inactive surfactin synthetase apoform to cofactor containing holoform [53].

The regulation of surfactin biosynthesis is closely connected to other stationary phase induced phenomena and competence development pathway. *SrfA* expression is induced in the late exponential phase and is controlled by global regulatory mechanisms ComP-ComA and Spo0A-AbrB which sense and respond to nutritional stress and modulate the expression of a variety of genes. Natural competence defines the ability for exogenous DNA uptake. Remarkably, the *comS* gene involved in *B. subtilis* competence development is located within and out of frame of the *srfA* gene [51].

Surfactin resistance is at least to a certain extent provided by YerP, the first published example of a RND (resistance, nodulation and cell division) family of multidrug efflux pumps in Gram-positive bacteria. However, it was shown that other additional mechanisms that participate in the efflux of surfactin and the producer self-resistance should exist [49].

6. Potential applications of surfactins

Surfactin exhibits a wide range of interactions with target cell membranes and possesses potential for various medical applications. Besides its antifungal and antibacterial effect [31], surfactin can also inhibit fibrin clot formation [5], induces the formation of ion channels in lipid bilayer membranes [54], blocks the activity of cyclic adenosine monophosphate [32], inhibits platelet and spleen cytosolic phospholipase A2 (PLA2) [55] and exhibits antiviral [56] and antitumor activities [57].

Resistance is generally rare against all lipopeptides and the development of a well-defined resistance mechanism has been proposed to be unlikely [58]. Although there is only limited knowledge of the lipopeptide molecular mode of action, a few key properties have been described. A number of lipopeptides tend to aggregate into oligomers and micelles and can readily interact with the cytoplasmic membrane via their hydrophobic moieties [28,59]. Thus, these types of compounds with their unusual structures that act rapidly on membrane integrity rather than on other vital processes are a growing source of concern in modern medicine and might perhaps hold promise for the development of a new generation of antibiotics [60].

6.1. Antibacterial and anti-inflammatory effect

The finding of a role for surfactants in defense against infection and inflammation in the human body is not a new phenomenon. Pulmonary surfactant is a lipoprotein complex synthesized and secreted by the epithelial cells of lungs into the extracellular space, where it lowers the surface tension at the air/liquid interface of the lung and represents a key factor in host defense [61].

Moreover, some biosurfactants are suitable alternatives to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents. This is of particular importance at this point in time, when increasing numbers of drug-resistant pathogenic bacteria impose a constant threat and there is a need for some other lines of therapy [62]. Nowadays, resistance is comprised of the drugs of last resort,

including methicillin and vancomycin. These antibiotics are used not only in the therapy of nosocomial infections caused by enterococci and *Staphylococcus aureus* [63] but also in the therapy of community-acquired methicillin resistant *S. aureus* (caMRSA), which has recently been detected and which is much more aggressive than its hospital relatives because it has a particular preference for the young and healthy [64].

A promising example of an antimicrobial lipopeptide that has even reached the level of commercial development is daptomycin (Cubicin®) which was approved for the treatment of complicated skin and skin-structure infections in September 2003 by the FDA [4]. Daptomycin produced by *Streptomyces roseosporus* [65] has been shown to be highly active against multi-resistant bacteria such as methicillin-resistant *S. aureus* (MRSA) [66].

Recently, several studies have revealed the impact of surfactin on silencing the inflammatory effect of the lipopolysaccharide (LPS) interaction with eukaryotic cells. Compounds that inactivate LPS activity have the potential of being new anti-inflammatory agents. Surfactin inhibits the LPS-induced expression of inflammatory mediators (IL-1β and iNOS) [67] and reduces the plasma endotoxin, TNF-α and nitric oxide levels in response to septic shock in rats [68]. Surfactin was also shown to suppress the interaction of lipid A with LPS-binding protein (LBP) that mediates the transport of LPS to its receptors. Moreover, surfactin did not influence the viability of the tested eukaryotic cell lines [69].

6.2. Anti-Mycoplasma effect

Surfactin treatment of mammalian cells that had been contaminated with mycoplasmas improved the proliferation rates and led to changes in the cell morphology. In addition, the low cytotoxicity of surfactin to mammalian cells permitted specific inactivation of mycoplasmas without significantly damaging effects on cell metabolism in the culture [70]. A recent study confirmed surfactin potential to kill mycoplasma cells (MIC 25 μM) independently of the target cell concentration, which is a significant advantage over the mode of action of conventional antibiotics. In combination with enrofloxacin, surfactin exhibited a synergistic effect and resulted in mycoplasma-killing activity at about two orders of magnitude higher than those of the entire molecules used separately [71].

6.3. Surfactin role in surface colonization by pathogenes

Swarming motility and biofilm formation are the key actions in the colonization of a surface by bacteria and increase the likelihood of nosocomial infections. Various nosocomial infections such as those related to the use of central venous catheters, urinary catheters, prosthetic heart valves, voice prostheses and orthopedic devices are clearly associated with biofilms that adhere to the biomaterial surface. These infections share common characteristics even though the microbial causes and host sites vary greatly [72]. The most important of these features is that bacteria in biofilms are highly resistant to antibiotics and so they evade host defenses and withstand antimicrobial chemotherapy [73]. Biosurfactants such as surfactin have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or the infection sites. Surfactin decreases the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enterica*, *Eschericha coli* and *Proteus mirabilis* in polyvinyl chloride wells, as well as vinyl urethral catheters. Precoating the catheters by running the surfactin solution through them before inoculation with media was just as effective as including surfactin in the growth medium. Given the importance of opportunistic infections with *Salmonella* species, including the urinary tract of AIDS patients, these results have potential for practical application [74].

6.4. Anti-viral activity

Surfactin is active against several viruses including Semliki Forest virus, herpes simplex virus (HSV-1 a HSV-2), vesicular stomatitis virus, simian immunodeficiency virus, feline calicivirus and murine encephalomyocarditis virus. The inactivation of enveloped viruses, especially herpes viruses and retroviruses, was significantly more efficient than that of non-enveloped viruses. This suggests that the antiviral action of surfactin is primarily due to a physicochemical interaction between the membrane active surfactant and the virus lipid membrane [75]. One of the important factors for virus inactivation is the number of carbon atoms of the fatty acid chain of surfactin. With increasing fatty acid hydrophobicity the virus inactivation capacity increases. During inactivation, surfactin incorporates into the lipid bilayer inducing a complete disintegration of the envelope which contains the viral proteins involved in virus adsorption and penetration. Its absence accounts for the loss of viral infectivity [56].

6.5. Antitumor activity

Surfactin has also been reported to have an antitumor activity against Ehrlich's ascite carcinoma cells [57]. A recent study on the effect of surfactin on the proliferation of a human colon carcinoma cell line showed that surfactin strongly blocked the cell proliferation. The growth inhibition by surfactin was due to apoptosis induction and cell cycle arrest via the suppression of cell survival regulating signals such as ERK and PI3K/Akt [76].

6.6. Thrombolytic activity

The plasminogen-plasmin system involved in blood clot dissolution participates in a variety of physiological and pathological processes requiring localized proteolysis. Plasminogen is activated proteolytically by urokinase-type plasminogen activator (u-PA), which is initially secreted as a zymogen prourokinase (pro-u-PA). Along with activation by u-PA, the plasminogen itself has an activation mechanism involving conformational change. The reciprocal activation of plasminogen and prourokinase is an important mechanism in the initiation and propagation of local fibrinolytic activity. Surfactin at concentrations of 3 – 20 μM enhances the activation of prourokinase as well as the conformational change in the plasminogen, leading to increased fibrinolysis *in vitro* and *in vivo* [77]. In a rat pulmonary embolism model, surfactin increased plasma clot lysis when injected in combination with prourokinase [78]. Surfactin is also able to prevent a platelet aggregation leading to the inhibition of additional fibrin clot formation, and to enhance fibrinolysis with the facilitated diffusion of fibrinolytic agents [79]. The anti-platelet activity of surfactin is not due to its detergent effect but due to its action on the downstream signaling pathways [80]. These results suggest a possible use for surfactin in urgent thrombolytic therapy related to pulmonary, myocardial and cerebral disorders. Moreover, surfactin has advantages over other available thrombolytical agents because it has fewer side effects and, hence, has potential for long-term use.

6.7. Toxicity

A possible toxicity (e.g. hemolytic activity) of the surfactin molecule constitutes a drawback for its medical applications at present. Hemolytic activity of surfactin was seen at high concentrations of about 40 μM to 60 μM [81]. On the other hand, the lowest surfactin concentration that completely inhibited the growth of mycoplasmas after 48 h (MIC) is 25 μM [71] and 30 μM surfactin treatment for 24 h displayed significant anti-proliferative activity of human colon cancer cells [76].

The LD_{50} (Lethal Dose, 50%; the dose required to kill half the members of a tested population) of surfactin is at > 100 mg/kg, i.v. in mice. Oral intake up to 10 mg of the lipopeptide in long-term application did not show apparent toxicities [68]. Surfactin did not influence the viability of HUVEC (human umbilical vein endothelial cells) up to 30 μg/ml after 24 hours. Surfactin was also regarded less toxic than other surfactants as judged from the results of an acute toxicity study in mice [69] and also as a safer anti-endotoxin agent in comparison with polymyxin B [68].

Another possibility to reduce the surfactin toxicity is minor alterations in the chemical structure of the molecule that may lead to a dramatic modification in the toxicity profile of any compound. Genetic engineering of the surfactin synthetase resulted in the production of a novel antimicrobial agent. Reduced toxicity against erythrocytes concomitant with an increase in growth inhibition of bacterial cells was observed [82]. Similarly, organic synthesis offers to produce linear forms of surfactin which are not hemolytic and can even protect red blood cells against the action of other detergents. Another potential use of linear surfactin analogs is the incorporation into cyclic surfactin in order to take advantage of its protective effect [83] or the delivery of cyclic surfactin in a liposome of a specific phospholipid constitution into different kinds of target cells [84]. Thus, similar surfactin derivatives may exhibit reduced toxicity against eukaryotic cells, which could improve their therapeutic applications.

7. Conclusions

Biosurfactants as membrane active agents represent a promising alternative to synthetic medicine and antimicrobial compounds, and could be used as effective therapeutics, especially at time when drug resistance among causal organisms for many life-threatening diseases is on the rise. In spite of the immense potential of biosurfactants in biomedicine, their usage still remains limited, possibly due to their high production cost, scarce knowledge of their molecular mode of action and an unclear toxicity towards the human organism. Last but not least, the mechanism of surfactin resistance should also be elucidated.

Further investigation on human cells and natural microbiota needs to be carried out to validate the use of biosurfactants in biomedical and health-related areas. Nevertheless, it is only a matter of time before the full potential of biosurfactants is fully exploited and used in medical science.

References

[1] Boman H.G., Peptide antibiotics and their role in innate immunity, Annu. Rev. Immunol., 1995, 13, 61-92
[2] Zasloff M., Innate immunity, antimicrobial peptides, and protection of the oral cavity, Lancet, 2002, 360, 1116-7
[3] Zhang L., Falla T.J., Cationic antimicrobial peptides – An update, Expert. Opin. Invest. Drugs, 2004, 13, 97-106
[4] Giuliani A., Pirri G., Fabiole Nicoletto S., Antimicrobial peptides: an overview of a promising class of therapeutics, Central European Journal of Biology, 2007, 2, 1-33
[5] Arima K., Kakinuma A., Tamura G., Surfactin, a crystalline peptidelipid surfactant produced by Bacillus subtilis: isolation, characterization and its inhibition of fibrin clot formation, Biochem. Biophys. Res. Commun., 1968, 31, 488-494
[6] Kakinuma A., Hori M., Isono M., Tamura G., Arima K., Determination of amino acid sequence in surfactin, a crystalline peptidolipid surfactant produced by Bacillus subtilis, Agric. Biol. Chem., 1969, 33, 971-997
[7] Rosenberg E., Ron E.Z., High- and low-molecular-mass microbial surfactants, Appl. Microbiol. Biotechnol., 1999, 52, 154-162
[8] Mulligan C.N., Environmental applications for biosurfactants, Environ. Pollut., 2005, 133, 183-198
[9] Becher P., Emulsions, theory and practice, 2nd ed., Reinhold Publishing, New York, 1965
[10] Cameotra S.S., Makkar R.S., Synthesis of biosurfactants in extreme conditions, Appl. Microbiol. Biotechnol., 1998, 50, 520-529
[11] Geiger T., Clarke S., Deamidation, isomerization, and racemization at asparaginyl and aspartyl residues in peptides. Succinimide-linked reactions that contribute to protein degradation, J. Biol. Chem., 1987, 262, 785-794
[12] Desai J.D., Banat I.M., Microbial production of surfactants and their commercial potential, Microbiol. Mol. Biol. Rev., 1997, 61, 47-64
[13] Kinsinger R.F., Kearns D.B., Hale M., Fall R., Genetic requirements for potassium ion-dependent colony spreading in Bacillus subtils, J. Bacteriol., 2005, 187, 8462-8469
[14] Julkowska D., Obuchowski M., Holland I.B., Séror S.J., Comparative analysis of the development of swarming communities of Bacillus subtilis 168 and a natural wild type: critical effects of surfactin and the composition of the medium, J. Bacteriol., 2005, 187, 65-76
[15] Stanley N.R., Lazazzera B.A, Environmental signals and regulatory pathways that influence biofilm formation, Mol. Microbiol., 2004, 52, 917-924
[16] Bonmatin J.M., Laprévote O., Peypoux F., Diversity among microbial cyclic lipopeptides: iturins and surfactins. Activity-structure relationship to design new bioactive agents, Comb. Chem. High Throughput Screen., 2003, 6, 541-556
[17] Kowall M., Vater J., Kluge B., Stein T., Franke P., Ziessow D., Separation and characterization of surfactin isoforms produced by Bacillus subtilis OKB 105, J. Colloid. Interface Sci., 1998, 204, 1-11
[18] Hue N., Serani L., Laprévote O., Structural investigation of cyclic peptidolipids from Bacillus subtilis by high energy tandem mass spectrometry, Rapid Commun. Mass Spectrom., 2001, 15, 203-209
[19] Bonmatin J.M., Genest M., Labbé H., Ptak M., Solution three-dimensional structure of surfactin: a cyclic lipopeptide studied by 1H-NMR, distance geometry, and molecular dynamics, Biopolymers, 1994, 34, 975-986
[20] Bonmatin J.M., Genest M., Labbé H., Grangemard I., Peypoux F., Maget-Dana R., et al., Production, isolation and characterization of [Leu4]- and [Ile4]-surfactins from Bacillus subtilis, Lett. Peptide Sci., 1995, 2, 41-47
[21] Tsan P., Volpon L., Besson F., Lancelin J.M., Structure and dynamics of surfactin studied by NMR in micellar media, J. Am. Chem. Soc., 2007, 129, 1968-77
[22] Peypoux F., Bonmatin J.M., Wallach J., Recent trends in the biochemistry of surfactin, Appl. Microbiol. Biotechnol., 1999, 51, 553-563
[23] Heerklotz H., Seelig J., Detergent-like action of the antibiotic peptide surfactin on lipid membranes, Biophys. J., 2001, 81, 1547-1554
[24] Bernheimer A.W., Avigad L.S., Nature and properties of a cytolytic agent produced by Bacillus subtilis, J. Gen. Microbiol., 1970, 6, 361-366
[25] Carrillo C., Teruel J.A., Aranda F.A., Ortiz A., Molecular mechanism of membrane permeabilization by the peptide antibiotic surfactin, Biochem. Biophys. Acta, 2003, 1611, 91-97
[26] Maget-Dana R., Ptak M., Interactions of surfactin with membrane models, Biophys. J., 1995, 68, 1937-1943
[27] Grau A., Gomez Fernandez J.C., Peypoux F., Ortiz A., A study on the interactions of surfactin with phospholipid vesicles, Biochim. Biophys. Acta, 1999, 1418, 307-319
[28] Heerklotz H., Seelig J., Leakage and lysis of lipid

membranes induced by the lipopeptide surfactin, Eur. Biophys. J., 2007, 36, 305-314
[29] Maget-Dana R., Ptak M., Interfacial properties of surfactin, J. Colloid Interface Sci., 1992, 153, 285-291
[30] Osman M., Høiland H., Holmsen H., Ishigami Y., Tuning micelles of a bioactive heptapeptide biosurfactant via extrinsically induced conformational transition of surfactin assembly, J. Pept. Sci., 1998, 4, 449- 458
[31] Thimon L., Peypoux F., Maget-Dana R., Roux B., Michel G., Interactions of bioactive lipopeptides, iturin A and surfactin from Bacillus subtilis, Biotechnol. Appl. Biochem., 1992, 16, 144-151
[32] Hosono K., Suzuki H., Acylpeptides, the inhibition of cyclic adenosine 3',5'-monophosphate phosphodiesterase. III. Inhibition of cyclic AMP phosphodiesterase, J. Antibiot., 1983, 36, 679-683
[33] Morikawa M., Hirata Y., Imanaka T., A study on the structure-function relationship of lipopeptide biosurfactants, Biochim. Biophys. Acta, 2000, 1488, 211-218
[34] Lipmann F., Gevers W., Kleinkauf H., Roskoski, R. Jr., Polypeptide synthesis on protein templates: the enzymatic synthesis of gramicidin S and tyrocidine, Adv. Enzymol. Relat. Areas Mol. Biol., 1971, 35, 1-34
[35] Sieber S.A., Marahiel M.A., Learning from nature's drug factories: nonribosomal synthesis of macrocyclic peptides, J. Bacteriol., 2003, 185, 7036-7043
[36] Kluge B., Vater J., Salnikow J., Eckart K., Studies on the biosynthesis of surfactin, a lipopeptide antibiotic from Bacillus subtilis ATCC 21332, FEBS Lett., 1988, 231, 107-110
[37] Nakano M.M., Zuber P., Molecular biology of antibiotic production in Bacillus, Biotechnology, 1990, 10, 223-240
[38] Vater J., Stein T., Vollenbroich D., Kruft V., Wittmann-Liebold B., P. Franke, et al., The modular organization of multifunctional peptide synthetases, J. Protein Chem., 1997, 16, 557-564
[39] Steller S., Sokoll A., Wilde C., Bernhard F., Franke P., Vater J., Initiation of surfactin biosynthesis and the role of the SrfD-thioesterase protein, Biochemistry, 2004, 43, 11331-11343
[40] Conti E., Stachelhaus T., Marahiel M.A, Brick P., Structural basis for the activation of phenylalanine in the non-ribosomal biosynthesis of gramicidin S, EMBO J., 1997, 16, 4174-4183
[41] Dieckmann R., Lee Y.O., van Liempt H., von Dohren H., Kleinkauf H., Expression of an active adenylate-forming domain of peptide synthetases corresponding to acyl-CoA-synthetases, FEBS Lett., 1995, 357, 212-216
[42] Weber T., Baumgartner R., Renner C., Marahiel M.A., Holak T.A., Solution structure of PCP, a prototype for the peptidyl carrier domains of modular peptide synthetases, Structure, 2000, 8, 407-418
[43] Keating T.A., Marshall C.G., Walsh C.T., Keating A.E., The structure of VibH represents nonribosomal peptide synthetase condensation, cyclization and epimerization domains, Nat. Struct. Biol., 2002, 9, 522-526
[44] Belshaw P.J., Walsh C.T., Stachelhaus T., Aminoacyl-CoAs as probes of condensation domain selectivity in nonribosomal peptide synthesis, Science, 1999, 284, 486-489
[45] Schwarzer D., Mootz H.D., Linne U., Marahiel M.A., Regeneration of misprimed nonribosomal peptide synthetases by type II thioesterases, Proc. Natl. Acad. Sci. U.S.A., 2002, 99, 14083-14088
[46] Bruner S.D., Weber T., Kohli R.M., Schwarzer D., Marahiel M.A., Walsh C.T., et al., Structural basis for the cyclization of the lipopeptide antibiotic surfactin by the thioesterase domain SrfTE, Structure, 2002, 10, 301-310
[47] Tseng C.C., Bruner S.D., Kohli R.M., Marahiel M.A., Walsh C.T., Siber S.A., Characterization of the surfactin synthetase C-terminal thioesterase domain as a cyclic depsipeptide synthese, Biochemistry, 2002, 41, 13350-13359
[48] Linne U., Marahiel M.A., Control of directionality in nonribosomal peptide synthesis: role of the condensation domain in preventing misinitiation and timing of epimerization, Biochemistry, 2000, 39, 10439-10447
[49] Tsuge K., Ohata Y., Shoda M., Gene yerP, involved in surfactin self-resistance in Bacillus subtilis, Antimicrob. Agents Chemother., 2001, 45, 3566-3573
[50] Guenzi E., Galli G., Grgurina I., Pace E., Ferranti P., Grandi G., Coordinate transcription and physical linkage of domains in surfactin synthetase are not essential for proper assembly and ctivity of the multienzyme copmlex, J. Biol. Chem., 1998, 273, 14403-14410
[51] Hamoen L.W., Venema G., Kuipers O.P., Controlling competence in Bacillus subtilis: shared use of regulators, Microbiology, 2003, 149, 9-17
[52] Nakano M.M., Corbell N., Besson J., Zuber P., Isolation and charcterization of sfp: a gene that functions in the production of the lipopeptide biosurfactant, surfactin, in Bacillus subtilis, J. Bacteriol., 1992, 182, 3274-3277
[53] Lambalot R.H., Gehring A.M., Flugel R.S., Zuber

P., LaCelle M., Marahiel M.A., et al., A new enzyme superfamily - the phosphopantetheinyl transferases, Chem Biol., 1996, 3, 923-936

[54] Sheppard J.D., Jumarie C., Cooper D.G., Laprade R., Ionic channels induced by surfactin in planar lipid bilayer membranes, Biochim. Biophys. Acta, 1991, 26, 13-23

[55] Kim K., Jung S.Y., Lee D.K., Jung J.K., Park J.K., Kim D.K., et al., Suppression of inflammatory responses by surfactin, a selective inhibitor of platelet cytosolic phospholipase A2, Biochem. Pharmacol., 1998, 55, 975-985

[56] Kracht M., Rokos H., Ozel M., Kowall M., Pauli G., Vater J., Antiviral and hemolytic activities of surfactin isoforms and their methyl ester derivatives, J. Antibiot., 1999, 52, 613-619.

[57] Kameda Y., Oira S., Matsui K., Kanatomo S., Hase T., Antitumor activity of Bacillus natto. V. Isolation and characterization of surfactin in the culture medium of Bacillus natto KMD 2311, Chem. Pharm. Bull., 1974, 22, 938-944

[58] Barry A.L., Fuchs P.C., Brown S.D., Evaluation of daptomycin susceptibility testing by Etest and the effect of different batches of media, J. Antimicrob. Chemother., 2001, 48, 557-561

[59] Rotondi K.S., Gierasch L.M., A well-defined amphipathic conformation for the calcium-free cyclic lipopeptide antibiotic, daptomycin, in aqueous solution, Biopolymers, 2005, 80, 374-385

[60] Goldberg J., Cyclic peptide antibiotics; self-assembly required, Trends Microbiol., 2001, 9, 412

[61] Wright J.R., Pulmonary surfactant: a front line of lung host defense, J. Clin. Invest., 2003, 111, 1453-1455

[62] Singh P., Cameotra S.S., Potential applications of microbial surfactants in biomedical sciences, Trends Biotechnol., 2004, 22, 142-146

[63] Yoneyama H., Katsumata R., Antibiotic resistance in bacteria and its future for novel antibiotic development, Biosci. Biotechnol. Biochem., 2006, 70, 1060-1075

[64] Hadley C., Overcoming resistance, EMBO Rep., 2004, 5, 550-552

[65] McHenney M.A., Baltz R.H., Gene transfer and transposition mutagenesis in Streptomyces roseosporus: mapping of insertions that influence daptomycin or pigment production, Microbiology, 1996, 142, 2363-2373

[66] Tally F.P., De Bruin M.F., Development of daptomycin for gram-positive infections, J. Antimicrob. Chemother., 2000, 46, 523-526

[67] Hwang M.H., Lim J.H., Yun H.I., Rhee M.H., Cho J.Y., Hsu W.H., et al., Surfactin C inhibits the lipopolysaccharide-induced transcription of interleukin-1beta and inducible nitric oxide synthase and nitric oxide production in murine RAW 264.7 cells, Biotechnol. Lett., 2005, 27, 1605-1608

[68] Hwang Y.H., Park B.K., Lim J.H., Kim M.S., Park S.C., Hwang M.H., et al., Lipopolysaccharide-binding and neutralizing activities of surfactin C in experimental models of septic shock, Eur. J. Pharmacol., 2007, 556, 166-171

[69] Takahashi T., Ohno O., Ikeda Y., Sawa R., Homma Y., Igarashi M., et al., Inhibition of lipopolysaccharide activity by a bacterial cyclic lipopeptide surfactin, J. Antibiot., 2006, 59, 35-43

[70] Vollenbroich D., Pauli G., Ozel M., Vater J., Antimycoplasma properties and application in cell culture of surfactin, a lipopeptide antibiotic from Bacillus subtilis, Appl. Environ. Microbiol., 1997, 63, 44-49

[71] Fassi Fehri L., Wroblewski H., Blanchard A., Activities of antimicrobial peptides and synergy with enrofloxacin against Mycoplasma pulmonis, Antimicrob. Agents Chemother., 2007, 51, 468-74

[72] Rodrigues L., Banat I.M., Teixeira J., Oliveira R., Biosurfactants: potential applications in medicine, J. Antimicrob. Chemother., 2006, 57, 609-618

[73] Morikawa M., Beneficial biofilm formation by industrial bacteria Bacillus subtilis and related species, J. Biosci. Bioeng., 2006, 101, 1-8

[74] Mireles 2nd J.R., Toguchi A., Harshey R.M., Salmonella enterica serovar typhimurium swarming mutants with altered biofilm-forming abilities: surfactin inhibits biofilm formation, J. Bacteriol., 2001, 183, 5848-5854

[75] Vollenbroich D., Ozel M., Vater J., Kamp R.M., Pauli G., Mechanism of inactivation of enveloped viruses by the biosurfactant surfactin from Bacillus subtilis, Biologicals, 1997, 25, 289-297

[76] Kim S.Y., Kim J.Y., Kim S.H., Bae H.J., Yi H., Yoon S.H., et al., Surfactin from Bacillus subtilis displays anti-proliferative effect via apoptosis induction, cell cycle arrest and survival signaling suppression, FEBS Lett., 2007, 581, 865-871

[77] Kikuchi T., Hasumi K., Enhancement of plasminogen activation by surfactin C: augmentation of fibrinolysis in vitro and in vivo, Biochim. Biophys. Acta, 2002, 29, 234-245

[78] Kikuchi T., Hasumi K., Enhancement of reciprocal activation of prourokinase and plasminogen by the bacterial lipopeptide surfactins and iturin Cs, J. Antibiot., 2003, 56, 34-37

[79] Lim J.H., Park B.K., Kim M.S., Hwang M.H., Rhee M.H., Park S.C., et al., The anti-thrombotic activity of surfactins, J. Vet. Sci., 2005, 6, 353-355

[80] Kim S.D., Park S.K., Cho J.Y., Park H.J., Lim J.H., Yun H.I., et al., Surfactin C inhibits platelet aggregation, J. Pharm. Pharmacol., 2006, 58, 867-870

[81] Dehghan-Noudeh G., Housaindokht M., Fazly Bazzaz B.S., Isolation, characterization, and investigation of surface and hemolytic activities of a lipopeptide biosurfactant produced by Bacillus subtilis ATCC 6633, J. Microbiol., 2005, 43, 272-276

[82] Symmank H., Franke P., Saenger W., Bernhard F., Modification of biologically active peptides: production of a novel lipohexapeptide after engineering of Bacillus subtilis surfactin synthetase, Protein Eng., 2002, 15, 913-921

[83] Dufour S., Deleu M., Nott K., Wathelet B., Thonart P., Paquot M., Hemolytic activity of new linear surfactin analogs in relation to their physico-chemical properties, Biochim. Biophys. Acta, 2005, 1726, 87-95

[84] Bouffioux O., Berquand A., Eeman M., Paquot M., Dufrêne Y.F., Brasseur R., et al., Molecular organization of surfactin-phospholipid monolayers: effect of phospholipid chain length and polar head, Biochim. Biophys. Acta, 2007, 1768, 1758-1768