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Historical perspective

Amino acid–based surfactants: New antimicrobial agents

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ABSTRACT

The rapid increase of drug resistant bacteria makes necessary the development of new antimicrobial agents. Synthetic amino acid-based surfactants constitute a promising alternative to conventional antimicrobial compounds given that they can be prepared from renewable raw materials. In this review, we discuss the structural features that promote antimicrobial activity of amino acid-based surfactants. Monocatenary, dicatenary and gemini surfactants that contain different amino acids on the polar head and show activity against bacteria are revised. The synthesis and basic physico-chemical properties have also been included.

Contents

1. Introduction

The rapid increase of multiple drug resistant bacteria and fungi poses a serious threat to society [1]. For example, in the United States and in the United Kingdom, 40–60% of nosocomial *Staphylococcus aureus* strains are methicillin-resistant (MRSA) [2] and more deaths are associated with MRSA than with methicillin-sensitive strains [3]. Therefore, there is an urgent necessity to design new antimicrobial compounds that impede the development of acquired resistance. One possible strategy is the preparation of compounds with novel modes of action and different targets compared to existing antibiotics.

Cationic antimicrobial peptides (AMPs) are emerging as potent antimicrobial agents. Produced by all types of living organisms, AMPs are key components of the innate immune system and are active against invading pathogens, including bacteria, fungi and yeast [4]. These antimicrobials consist of 12–50 amino acids that show a net positive charge at physiological pH and a hydrophobic bulk. An optimal association between the cationic charge and the hydrophobic part of the molecule was found to be crucial for the antimicrobial activity of these compounds [5]. Importantly, AMPs usually work through relatively non-specific modes of action, in contrast with conventional antibiotics, which target specific enzymes or DNA. Instead of targeting a single

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molecule or process, they associate with cellular membranes, resulting in depolarization, lysis, and cell death, probably due to their successful incorporation into the hydrophobic lipid bilayer [6]. This hampers the development of bacterial resistance against their activity. However, in spite of the excellent antimicrobial performance of AMPs, there are some drawbacks in their application [7]: a) their chemical synthesis entails a high production cost, b) linear peptides are often easily proteolytically degraded, and c) they are not generally cell-specific and are also toxic to mammalian cells.

Native lipopeptides composed of an aliphatic acid attached to a short peptide portion of six or seven amino acids are also effective antimicrobial agents. Like AMPS, they act via perturbation of the bacterial membrane [8], and have the same disadvantage in that they are toxic to mammalian cells due to a lack of selectivity [9].

Classic cationic surfactants derived from quaternary ammonium groups are also applied for their antimicrobial properties [10]. In fact, these surfactants have been used for well over half a century as antiseptics together with products such as dressing, catheters and sutures. By reducing hospital infections, cationic antimicrobials diminish the need for antibiotics, which has had a positive impact on antibiotic resistance development. Moreover, despite their widespread usage, no apparent reduction in their effectiveness has been observed. Nevertheless, they are not suitable for biomedical applications due to their haemolytic activity and cytotoxicity [11]. Their use is also seriously questioned from an environmental point of view as they are not readily biodegradable and are toxic to aquatic organisms [12]. Insufficient biodegradation of chemical compounds increases their toxicity, given that aquatic organisms have more time in contact with them. Laboratory studies have shown that dimethyldidecyl ammonium chloride is hydrolytically stable and resistant to microbial degradation and the predicted half-lives in aquatic environments are equal to or longer than 3 years [13].

Nowadays, synthetic cationic amino acid-based surfactants are being explored as promising alternatives to conventional antimicrobial agents. Structurally, these compounds can be considered as analogues of native lipopeptides since they are cationic amphiphiles consisting of one or two amino acids linked to a hydrophobic moiety. They therefore share the same mode of action against microorganisms and low susceptibility to inducing resistance.

The interaction of cationic amphiphiles with microorganisms involves two main steps. Firstly, the amphiphile becomes attached to the target membrane, a process governed by electrostatic interactions between the positively charged polar head of the surfactants and the negatively charged molecules of the bacterial membranes (lipopolysaccharides in Gram-negative and lipoteichoic acid in Gram-positive bacteria) [14]. These electrostatic interactions are also entropically favoured due to the release of counterions. Subsequently, the hydrophobic alkyl chain of cationic amphiphiles interacts with the lipid bilayers of membranes, modifying the membrane architecture and promoting the transport of intracellular constituents across the cell membranes [15]. In this second step, an optimum relationship between the hydrophobicity and polarity of the surfactant is necessary to facilitate the diffusion of the surfactant in the non-polar environment of lipid bilayers. This mechanism explains the activity of cationic surfactants, generally more potent against Gram-positive bacteria, which contain high amounts of negatively charged lipids. Most of them do not show antifungal activity because the negative charge density at the cell membrane in these microorganisms is lower than in bacteria [16].

The high number of different types of amino acids, as well as their diverse nature (polar, non polar, acidic, basic), allows a wide range of cationic amphiphile structures with diversified specifications to be designed. Furthermore, given their simple structure, consisting of one or two amino acids linked to an aliphatic chain, their synthesis does not involve many steps. Consequently, their manufacturing cost is economically viable and they can be prepared in accordance with the current environmental regulations (use of renewable starting materials with a low volume of hazardous solvents). In general, these kinds of surfactants can be considered as readily biodegradable compounds. One of the major advantages of antimicrobial amino-acid surfactants is their double functionality: as well as featuring the typical properties of surface active molecules (forming molecular aggregates as micelles or vesicles and thereby reducing surface tension), they can also act as antimicrobial agents.

2. Structure, synthesis and properties of antimicrobial amino acid-based surfactants

2.1. Single chain surfactants with one amino acid

Amino acid-based surfactants constitute an interesting alternative to conventional synthetic surfactants, bearing some of the fundamental requirements for industrial development: (a) multifunctionality, (b) low toxicity, (c) renewable sources of raw materials, (d) biodegradability and (e) simple synthesis.

Amino acids have at least two functional groups, the carboxylic group and the amino group. These compounds can be easily converted to single chain surfactants with a reactive molecule bearing a hydrophobic chain, such as fatty acids, fatty esters, fatty amines and fatty alcohols. The hydrophobic chain can be introduced into the amino acid structure through ester alkyl or amide linkages (Fig. 1).

Amino acids with reactive side chains such as lysine or arginine offer additional opportunities for the molecular design of monocatenary surfactants. From an economical and environmental point of view, single chain surfactants with only one amino acid on the polar head are highly attractive compounds, since they can be easily prepared.

2.1.1. Arginine

Due to the presence of a guanidine group, the arginine amino acid is an excellent raw material to prepare surfactants with antimicrobial activity. The literature describes a significant number of compounds with excellent antiseptic and pharmacological behaviour whose common feature is strongly basic groups of the guanidine type attached to a fairly large lipophilic molecule [17]. Our group has developed different synthetic routes (chemical, enzymatic or a combination of both methodologies) to prepare a wide range of single chain arginine structures (Fig. 2) [18].

Long chain N^{α}-acyl arginine methyl or ethyl ester surfactants (Fig. 2, series 1) were synthesized by the condensation of one hydrophobic group to the α-amino group of the arginine methyl or ethyl ester. The guanidine side chain of arginine is an extremely strong base, which in an unsubstituted state remains protonated under normal conditions for α-acylation [19]. It is therefore possible to work with an unprotected arginine side chain by carefully controlling the pH, and dicyclohexyl carbodiimide can be used to condense the fatty acid [20]. This method allowed the use of very mild experimental conditions, thereby avoiding racemization and the formation of undesirable by-products. The long fatty acyl chain can also be introduced with the corresponding acid chloride by using a mixture of water/acetone as the solvent or only water [21].

Arginine-n-alkyl amide dihydrochlorides (Fig. 2, series 2) and arginine-O-alkyl ester dihydrochlorides (Fig. 2, series 3) contain two positively charged groups in the polar head, one in the primary amine and the second in the guanidine function. Series 2 was first prepared by chemical procedures involving the condensation of the carboxylic group of Boc-protected arginine with the corresponding long chain alkylamine [22]. After that, an enzymatic procedure that allowed the preparation of series 2 and 3 was described [23]. Papain (from *Carica papaya*) deposited onto polyamide was found to be the best biocatalyst configuration for the formation of amide (series 2) and ester (series 3) bonds between Cbz-arginine methyl ester and various long chain amines and fatty alcohols. The sodium salts of N^{α}-acyl arginine (Fig. 2, series 4) are amphoteric surfactants that contain one positive charge (guanidine group) and one negative charge (carboxylic group) on the