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Guanidino groups greatly enhance the action of antimicrobial peptidomimetics against bacterial cytoplasmic membranes

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ABSTRACT

Antimicrobial peptides or their synthetic mimics are a promising class of potential new antibiotics. Herein we assess the effect of the type of cationic side chain (i.e., guanidino vs. amino groups) on the membrane perturbing mechanism of antimicrobial α-peptide–β-peptoid chimeras. Two separate Langmuir monolayers composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylglycerol (DPPG) and lipopolysaccharide Kdo2-lipid A were applied to model the outer membranes of Gram-positive and Gram-negative bacteria, respectively. We report the results of the measurements using an array of techniques, including high-resolution synchrotron surface X-ray scattering, epifluorescence microscopy, and in vitro antimicrobial activity to study the molecular mechanisms of peptidomimetic interaction with bacterial membranes. We found guanidino group-containing chimeras to exhibit greater disruptive activity on DPPG monolayers than the amino group-containing analogues. However, this effect was not observed for lipopolysaccharide monolayers where the difference was negligible. Furthermore, the addition of the nitrobenzoxadiazole fluorophore did not reduce the insertion activity of these antimicrobials into both model membrane systems examined, which may be useful for future cellular localization studies.

1. Introduction

Antimicrobial peptides (AMPs) are ubiquitous in nature; present in virtually all organisms they serve as endogenous antibiotics through the innate immune response [1,2]. Members of this class of compounds have been studied extensively due to their potential as promising alternative antibiotics to treat disease caused by the growing number of resistant pathogenic microbes [1–4]. It is generally believed that AMPs exert their direct killing of invading pathogens by selectively interacting with the negatively charged bacterial surfaces over the globally neutral (zwitterionic) eukaryotic cell membranes. The mechanism by which the membranes are permeated is not completely understood, and several models have been proposed based on studies conducted with various peptidic structures [1]. Moreover, recent studies have shown that some of these chemotypes are endowed with additional intracellular modes of action such as interference with cell wall biosynthesis or immunomodulatory effects [5–9]. These findings complicate the understanding of this class of compounds even further and have called for the use of a perhaps more appropriate class designation, host-defense peptides (HDPs) [3].

Despite their diversity in amino acid sequence, lipophilicity and secondary structure [10], most HDPs share common features including positive net charge and generally amphipathic nature, separating hydrophilic and hydrophobic residues to the opposite faces of the molecule [11–13]. Typically, positive net charge of naturally occurring peptides is contributed by the guanidino groups of the arginine (Arg) [14,15] and/or amino groups of the lysine (Lys) residues [16–18]. Both Arg and Lys side chains are generally thought to promote the initial long range electrostatic attractive forces that guide antimicrobials towards the negatively charged bacterial membranes [19]. However, guanidino groups have higher acid dissociation constant (pK_a) due to efficient resonance stabilization of the charged protonated state together with efficient solvation in water, which makes them stronger bases and, thus, better suited for stable electrostatic interactions with the negatively charged phosphodiester and phosphomonoester groups

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of phospholipids [20–24]. Examples of naturally occurring AMPs containing arginine rather than lysine residues include several members of the cathelicidin family, such as indolicidin and tritrpticin [25,26]. Also, in peptides having high content of both arginine and lysine residues such as the defensins, these residues are not randomly distributed within their sequence and their ordering implies a significance greater than just a net positive charge [27]. Muhle and Tam [28] found that Arg-to-Lys substitution in a cyclic disulfide-stabilized peptide decreased activity against Gram-negative bacteria. Nakase et al. demonstrated improved membrane permeability of antimicrobial peptide (RLA) with lysine substituted by arginine [29]. Other studies have shown that for lactoferricin B and bactenecin 5, which have no hemolytic activity, the replacement of arginine for lysine reduced antibacterial activity [30]. So, the incorporation of guanidino groups into the peptide side chains may have its appeal in drug design [31–33].

However, there are concerns related to the use of α-peptides in a clinical setting due to their high cost of manufacturing [34] and inherent susceptibility to proteases [35], which has led to numerous studies aimed at mimicry of peptides using non-natural compounds. Thus, a variety of classes such as β-peptides [36–38], oligoureas [39], arylamides [40,41], *N*-substituted oligoglycines (peptoids) [42–44], cyclic D,L-α-peptides [45–47], hybrid peptidomimetics [33,48–50], and polymers [51–53] have been designed to mimic the function of AMPs.

α-Peptide–β-peptoid chimeras represent a distinct class of peptidomimetics with backbone composed of alternating peptide and β-peptoid residues [33,50,54–56]. In the present study we elucidate the role of the cation type on the antimicrobial properties of this type of synthetic AMP mimic using two α-peptide–β-peptoid chimeras ($K\beta N_{spe}$ and $R\beta N_{spe}$), which differ from each other solely in the identity of cationic functionality [amine (lysine) vs. guanidino group (homoarginine)]. In addition, because fluorophore-labeled analogues of AMPs, which retain antimicrobial activity, constitute powerful tools for studying mechanisms of action and cellular localization, we also prepared and evaluated nitrobenzoxadiazole (NBD)-labeled oligomers NBD-$K\beta N_{spe}$ and NBD-$R\beta N_{spe}$ (Fig. 1A).

Regardless of whether the primary mode of action is of a membrane-disrupting nature or entails perturbation of intracellular targets, the initial interaction between antimicrobial and bacteria involves the cell surface. A fundamental understanding of these lipid–antimicrobial interactions is therefore important for the future design of improved antibiotics for potential clinical use. Since cell membranes have a complex structure and are currently not applicable for highly sensitive surface X-ray scattering methods, the model systems are generally employed to undertake detailed mechanistic studies of membrane-associated processes [57–61]. Previously, the membrane-destabilizing effects of the α-peptide–β-peptoid chimeras have only been investigated in model liposomes prepared from phosphatidylcholine (PC), a phospholipid found predominantly in eukaryotic cells [55]. However, PC-containing systems do not adequately represent bacterial envelope, and furthermore, these compounds have not been investigated using sensitive X-ray methods before.

In order to model the outer surface of Gram-positive and Gram-negative bacteria we have employed insertion assay experiments on two separate Langmuir monolayers composed of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol (DPPG) and truncated lipopolysaccharide (LPS) Kdo2-Lipid A, respectively (Fig. 1B). The reason behind this choice of lipids is that Kdo-2 lipid A constitutes the hydrophobic core of outer LPS envelope in most Gram-negative bacteria, while PGs are predominant anionic phospholipid species within cytoplasmic membranes of both Gram-negative and Gram-positive strains. This approach has been successfully used in conjunction with liquid surface X-ray scattering to study bacterial membrane lysis by human antimicrobial peptide LL-37 [60], protegrin-1 [57,62], gramicidin [63] and SMAP-29 [61] antimicrobial peptides as well as by peptide mimics [44,59,64,65].

2. Experimental section

2.1. Monolayer construction

Both DPPG and Kdo2-Lipid A were purchased from Avanti Polar Lipids (Alabaster, AL) and were used without further purification. To form the monolayer systems both DPPG and Kdo2-Lipid A were first dissolved in chloroform–methanol (65:25) at a concentration of 0.2 mg/mL. Using a microliter syringe (Hamilton) the solutions were

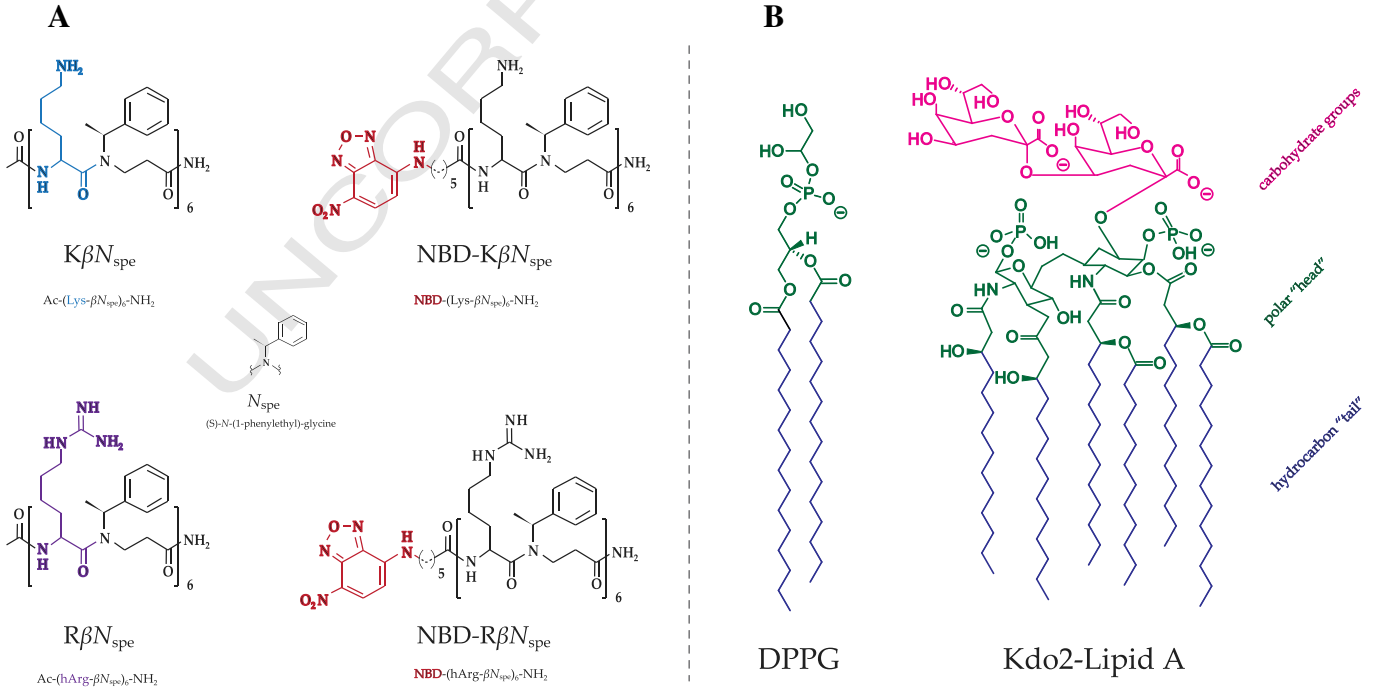

Fig. 1. Molecular structures of the tested chimeras (A) and lipids used for modeling bacterial cell membranes (B).

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