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Human neutrophil peptide 1 variants bearing arginine modified cationic side chains: Effects on membrane partitioning

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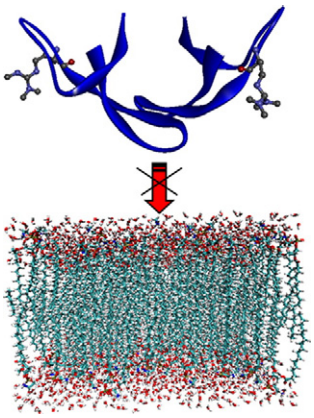
HIGHLIGHTS

- HNP-1 variants bearing Arginine modified side chains
- Model membranes mimicking composition of Gram-negative bacteria inner membrane
- Reduced interaction with negative charged model membranes
- Role of the Arg14 guanidino group for lipid interaction
- Comparison with native HNP-1 peptide at different peptide:lipid molar ratios

GRAPHICAL ABSTRACT



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ABSTRACT

α-Defensins (e.g. human neutrophil peptides, HNPs) have a broad spectrum bactericidal activity contributing to human innate immunity. The positive charge of amino acid side chains is responsible for the first interaction of cationic antimicrobial peptides with negatively charged bacterial membranes. α-Defensins contain a high content of Arg residues compared to Lys. In this paper, different peptide analogs including substitution of Arg-14 respectively with N^G–$N^{G'}$-asymmetric dimethyl-L-arginine (ADMA), N^G–$N^{G'}$-symmetric dimethyl-L-arginine (SDMA) and Lys (R14K and R15K) variants have been studied to test the role of Arg guanidino group and the localized cationic charge of Lys for interaction with lipid membranes. Our findings show that all the variants have a decreased disruptive activity against the bilayer. The methylated analogs show a reduction in membrane partitioning due to the lack of their ability to form hydrogen bonds. Comparison with the native HNP-1 peptide has been discussed.

Abbreviations: AMP, antimicrobial peptide; HNP-1, human neutrophil peptide 1; ADP, adenosine diphosphate; PMRTs, protein arginine methyltransferases; ADMA, N^G–$N^{G'}$-asymmetric dimethyl-L-arginine; SDMA, N^G–$N^{G'}$-symmetric dimethyl-L-arginine; MMA, N^G-monomethyl-L-arginine; POPG, 1-palmitoyl-2oleoyl-*sn*-glycerol-3-phosphoglycerol; POPE, 1-palmitoyl-2oleoyl-*sn*-glycerol-3-phosphoethanolamine; CL, 1,1′,2,2′-tetramyristoyl cardiolipin ammonium salt; PCSL, 1-acyl-2-[*n*-(4,4dimethyloxazolidinyl-N-oxyl)]stearoyl-*sn*-glycero-3-phosphocholine; MOPS, 3-(N-morpholino) propanesulfonic acid; LUVs, large unilamellar vesicles; CD, circular dichroism; EPR, electron paramagnetic resonance; PC, phopshatidylcholine.

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1. Introduction

Antimicrobial peptides (AMPs) have been isolated from plants, invertebrates, fish, insects, amphibians and mammals and constitute the first line of defense against bacteria and viruses [1,2]. The antimicrobial activity of some AMPs is based on membrane partitioning with phospholipid destabilization and pore formation, while other AMPs can inhibit more specific cellular targets such as protein expression and DNA replication [3,4]. Besides the composition of the phospholipid bilayer, the main factors that influence peptide–lipid interactions are the cationicity and the presence of hydrophobic residues in the peptide sequence [5].

Human neutrophil peptide 1 (HNP-1) is one of the most common human AMPs, expressed in the azurophil granules of neutrophils [6]. HNP-1 belongs to the class of α-defensin and it is structured in three β-sheets with three intramolecular disulfide bonds and a single β-hairpin. This peptide presents a total positive charge equal to +3 conferred by cationic arginines (Arg-5, Arg-14, Arg-15 and Arg-24) and anionic glutamic acid (Glu-13) [7,8]. Defensins are widely known to kill bacteria through electrostatic interactions with negatively-charged membranes. Arg residues are the most important for membrane interactions as the positively-charged side chains of cationic guanidino groups provide the initial long-range electrostatic attractive forces that guide the antimicrobial peptide toward the negatively-charged bacterial membranes. Arginines can also make electrostatic interactions with other residues (H-bonds, salt bridges and cationic–π contacts) important for structural stability [9,10].

In some inflammation diseases defensins can present an ADP-ribose unit linked to arginine residues that makes them inactive against pathogens. HNP-1 is ADP-ribosylated principally at Arg-14, which can be converted once modified, into an ornithine residue following a non-enzymatic hydrolytic reaction [11–13]. This post-translational modification to the guanidino group of arginine induces a reduction of peptide cationic charge. Along with ADP-ribosylation, also arginine methylation, catalyzed by a family of enzymes called protein arginine methyltransferases (PRMTs), represents another important protein post-translational modification. The change of arginine side chain guanidino groups is quantitatively one of the most extensive protein methylation reactions in mammalian cells [14]. Arginine is unique among amino acids as its guanidino group contains five potential hydrogen bond donors that are positioned for favorable interactions with biological hydrogen bond acceptors [15,16]. Thus, arginine modifications in proteins can readily modulate their binding interactions and thus regulate their physiological functions. The most prevalent methylated residue is N^G–$N^{G'}$-asymmetric dimethyl-L-arginine (ADMA), where two methyl groups are placed on one of the terminal nitrogen atoms of the guanidino group. Two other derivatives are formed, including the N^G–$N^{G'}$-symmetric dimethyl-L-arginine (SDMA) where one methyl group is placed on each of the terminal guanidino nitrogens and the mono-methylated derivative with a single methyl group on the terminal nitrogen atom, N^G-monomethyl-L-arginine (MMA) [15].

In this paper three different variants, including substitution of Arg-14 respectively with N^G–$N^{G'}$-asymmetric dimethyl-L-arginine (ADMA), N^G–$N^{G'}$-symmetric dimethyl-L-arginine (SDMA) and L-lysine (R14K) plus another variant replacing the Arg-15 with a L-lysine (R15K) have been studied for their interactions with lipid membranes. Although Arg and Lys have equivalent electropositive charges at neutral pH, only the Arg residues are thought to give selectivity to the peptides for preferential interactions with negatively charged bacterial membranes [16].

In this work, a biophysical study has been carried out to test the relevance of the dimethylated arginine residues (ADMA and SDMA) and lysine, incorporated into the most important sites of HNP-1, for partitioning into lipid membranes. All variants showed an altered capability of partition into lipid bilayer.

2. Material and methods

The dimethylated arginines (ADMA and SDMA) and lysine (R14K and R15K) peptide analogs of HNP-1 (Fig. 1) were obtained from Bachem (Bubendorf, CH) and used without further purification. All the variants used in the present study possess the same folding of native HNP-1 as confirmed by the Bachem data sheets.

The phospholipids for vesicle preparation, 1-palmitoyl-2oleoyl-*sn*-glycerol-3-phosphoglycerol (POPG), 1-palmitoyl-2oleoyl-*sn*-glycerol-3-phosphoethanolamine (POPE), 1,1′,2,2′-tetramyristoyl cardiolipin ammonium salt (CL), and spin-labeled phosphatidylcholine (1-acyl-2-[*n*-(4,4dimethyloxazolidinyl-N-oxyl)]stearoyl-*sn*-glycero-3-phosphocholine, *n*-PCSL, with n = 5, 7, 12) were obtained from Avanti Polar Lipids (Alabaster, AL). The buffer solution (pH 6.8) used in the experiments contains 50 mM of 3-(N-morpholino) propanesulfonic acid (MOPS) (Sigma Aldrich). MOPS is a buffer largely used for biological sample preparation due to its low salt content [17].

2.1. Liposome preparation

Liposomes were prepared by mixing 14 mM POPE, 12.9 mM POPG and 6.6 mM CL (POPE:POPG:CL 70:25:5 molar ratio), in agreement with the composition of lipids of Gram-negative bacterial inner membrane [18,19].

Lipids at the desired molar ratio dried down from chloroform stock solutions under a stream of nitrogen gas were then dried under vacuum for 1 h. The resulting lipid film was hydrated by adding 50 mM MOPS at pH 6.8 to reach a final concentration of about 50 mM phospholipids. Large unilamellar vesicles (LUVs) were prepared by freeze–thawing this lipid suspension five times followed by extrusion through 200 nm polycarbonate membrane filters using a Mini-Extruder syringe device (Avanti Polar Lipids). Final concentration of LUVs was determined using the Stewart phospholipids assay [20]. LUVs containing 1 mol of 5, 7, or 12-PCSL were prepared as described above. Different peptide:lipid molar ratios were prepared (1:250, 1:50, 1:30, 1:20, 1:15, 1:10 and 1:5) incubating each HNP-1 variant with LUVs in 50 mM MOPS buffer at pH 6.8 for at least 1 h.

2.2. Circular dichroism spectroscopy

Circular dichroism (CD) experiments were performed at room temperature on a Jasco CD-J-815 spectropolarimeter using a quartz cuvette with a path length of 1 mm. Peptides were suspended at 0.02 mM concentration in 50 mM MOPS at pH 6.8 with a constant or variable concentration of LUVs ranging from 0.1 to 5 mM. CD spectra were recorded from 190 to 250 nm and accumulated ten times to improve the signal-to-noise ratio. Baselines of either solvent or vesicular suspension without peptide were subtracted from each respective sample to calculate the peptide contribution [21].

2.3. Electron paramagnetic resonance spectroscopy

Electron paramagnetic resonance spectroscopy (EPR) spectra were recorded on a Bruker E500 ELEXSYS X-Band spectrometer equipped with a super-high-Q cavity at 303 K. Samples prepared for EPR measurements contained 0.4 mM of *n*-PCSL LUVs and a variable concentration of peptide ranging from 0.02 to 0.08 mM. Spectra were recorded using the following instrumental settings: 120 G sweep width; 100 kHz modulation frequency; 1.0 G modulation