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The interactions of antimicrobial peptides derived from lysozyme with model membrane systems☆

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Abstract

Two peptides, RAWVAWR-NH_2 and IVSDGNGMNAWVAWR-NH_2, derived from human and chicken lysozyme, respectively, exhibit antimicrobial activity. A comparison between the L-RAWVAWR, D-RAWVAWR, and the longer peptide has been carried out in membrane mimetic conditions to better understand how their interaction with lipid and detergent systems relates to the reported higher activity for the all L-peptide. Using CD and 2D ^{1}H NMR spectroscopy, the structures were studied with DPC and SDS micelles. Fluorescence spectroscopy was used to study peptide interactions with POPC and POPG vesicles and DOPC, DOPE, and DOPG mixed vesicle systems. Membrane–peptide interactions were also probed by ITC and DSC. The ability of fluorescein-labeled RAWVAWR to rapidly enter both *E. coli* and *Staphylococcus aureus* was visualized using confocal microscopy. Reflecting the bactericidal activity, the long peptide interacted very weakly with the lipids. The RAWVAWR-NH_2 peptides preferred lipids with negatively charged headgroups and interacted predominantly in the solvent–lipid interface, causing significant perturbation of membrane mimetics containing PG headgroups. Peptide structures determined by ^{1}H NMR indicated a well-ordered coiled structure for the short peptides and the C-terminus of the longer peptide. Using each technique, the two enantiomers of RAWVAWR-NH_2 interacted in an identical fashion with the lipids, indicating that any difference in activity in vivo is limited to interactions not involving the membrane lipids.

Keywords: Lysozyme; Antimicrobial peptide; Phospholipid; Membrane mimetic; Structure

1. Introduction

Over the last decade, the search for antibacterial agents has seen rapid growth with the discovery of numerous naturally occurring antimicrobial peptides. From the analysis of these unique peptides, the focus of much research has been centred upon exploiting the properties that selectively kill bacteria while leaving eukaryotic cells

Abbreviations: 1D, one dimensional; 2D, two dimensional; CD, circular dichroism; CFU, colony forming unit; CSI, chemical shift index; DSC, differential scanning calorimetry; nuclear magnetic resonance; DSS, sodium 3-(trimethylsilyl)-1-propanesulfonate; DPC, dodecylphosphocholine; DPPG, 1,2-dipalmitoyl-*sn*-3-phosphatidylglycerol; DPPC, dipalmitoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DO, dioleoyl; DOPC, dioleoylphosphatidylcholine; DOPG, dioleoylphosphatidylglycerol; DOPE, dioleoylphosphatidylethanolamine; HPLC, high performance liquid chromatography; ITC, isothermal titration calorimetry; LUVs, large unilamellar vesicles; MLVs, multilamellar vesicles; NAPB, sodium phosphate buffer; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser enhancement spectroscopy; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PO, 1-palmitoyl-2-oleoyl; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[(phospho-rac-(1-glycerol)] (sodium salt); rmsd, root-mean-square deviation; SDS, sodium dodecyl sulfate; TOCSY, total correlation spectroscopy

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unaffected [1]. To this end, a significant effort has been directed towards the understanding of specific interactions that enhance the bactericidal properties of a peptide [2–7]. Although antimicrobial peptides have been isolated from various species, many human sources of innate and putative antimicrobial peptides are continuously being discovered [8]. A novel branch of this work involves the discovery of antimicrobial peptides released upon the digestion or breakdown of proteins. Two examples are lactoferricin released from lactoferrin (for a recent review, see [9]) and various lysozyme peptides released from the proteolytic digest of lysozyme [10]. Other examples concern antimicrobial peptides from hemoglobin [11] or cathepsin [12].

Lysozyme has long been known for having the ability to disrupt many bacterial functions, including membrane structure [10,13–16]. This activity is not always related to its enzymatic activity; for example, inactivated lysozyme and specific peptides isolated from proteolytic digests of hen egg white lysozyme have been shown to exhibit antimicrobial activity against both Gram positive and Gram negative bacteria [10,17]. One of the peptides studied (IVSDGNGMNAWVAWR residues 98–112, see Fig. 1) was isolated and found to have varied activity. Yet, a refined search of the key portions exhibiting activity revealed that not all segments of this peptide retained antimicrobial properties. Ile98–Met105 was inactive while Asn106–Arg112 was weakly active. Furthermore, the C-terminal portion NAWRAWR showed improved antimicrobial activity by exchanging Asn with Arg. This modified segment corresponds to residues 107–113 of human lysozyme (see Fig. 1). In the earlier report, the natural all L-RAWVAWR peptide was more active than the synthetic all D-RAWVAWR peptide against *Serratia marcescens*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus lentus* [10]. These observations seemingly contradict many studies reporting that the replacement of L- with D-amino acids actually made little difference or created enhanced resistance to enzymatic degradation, thereby increasing the activity of the all D-peptides over all L-peptides (e.g. [18,19]). Other key aspects of this study were elucidated from specific amino acid substitutions in the peptide and indicated the necessity of two Arg as well two Trp residues for the peptide to maintain activity [10]. The notion that many peptides containing at least two Arg and two Trp have potent antimicrobial activity has recently been reviewed [9,20].

A Hen lysozyme

98 112

I V S D G N G M N A W V A W R

B V V R D P Q G I **R A W V A W R**

99 113

Human Lysozyme

Fig. 1. Amino acid sequences for hen lysozyme (A) and human lysozyme (B). Residues in bold are the subject of this study.

More recently, the scope of activity of the lysozyme-derived peptides was expanded to include the larger helix–loop–helix sections from chicken (Asp87–Arg114) and human (Asp87–Arg115) lysozyme in order to compare activity and membrane permeabilization [13]. This larger section of lysozyme also presented a wide range of bactericidal activity against both Gram positive and Gram negative bacteria. The most active segments in these studies were shown to be either the full helix–loop–helix or the latter sections Ala107–Arg114 in chicken and Arg107–Arg115 in human lysozyme. In both cases, the activity of the peptides centres around the cationic Trp containing segment (R)AWVAWR(NR).

Our current work involves a study of the longer peptide IVSDGNGMNAWVAWR-NH_2 from chicken lysozyme and the shorter, more active peptide RAWVAWR-NH_2 from human lysozyme, as well as a comparison between the L- and all D-forms of RAWVAWR-NH_2. These peptides have been studied under membrane mimetic conditions either with micelles or vesicles to obtain more detailed information regarding membrane binding and the resulting peptide secondary structure. We have combined ^{1}H NMR, fluorescence, CD, DSC, and ITC to provide a comprehensive and detailed analysis of the lysozyme peptide interactions with a variety of lipids with different headgroups. To complement these studies and put the peptide interactions in perspective of the overall cell penetration capabilities, we have also studied the location of fluorescein labeled peptides in both *E. coli* and *S. aureus*.

2. Methods and materials

2.1. Materials

The synthetic peptides RAWVAWR (L), RAWVAWR (D), and IVSDGNGMNAWVAWR (L) were synthesized at the peptide synthesis facility at the University of Waterloo (Waterloo, ON). All three peptides were amidated on the C-terminal end to remove the negative charge. DPPC, DPPG, DOPC, DOPE, DOPG, POPC, and POPG dissolved in chloroform were purchased from Avanti Polar Lipids (Alabaster, AL) and were used without further purification. Sodium dodecyl-d_{25} sulfate (SDS), dodecylphosphocholine-d_{38} (DPC), and D_2O were purchased from Cambridge Isotopes Laboratories, Inc. (Andover, MA). Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was purchased from MSD isotopes. Acrylamide and Tris were purchased from ICN Biomedicals Inc. (Aurora, OH). HEPES and MES were purchased from Sigma Chemical Co. (St. Louis, MO), and citric acid and NaCl were purchased from BDH Inc. (Toronto, ON). The N-terminally labeled fluorescein peptide RAWVAWR-NH_2 was purchased from UVic Protein Chem-