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Helical cationic antimicrobial peptide length and its impact on membrane disruption

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ABSTRACT

Cationic antimicrobial peptides (CAMPs) are important elements of innate immunity in higher organisms, representing an ancient defense mechanism against pathogenic bacteria. These peptides exhibit broad-spectrum antimicrobial activities, utilizing mechanisms that involve targeting bacterial membranes. Recently, a 34-residue CAMP (NA-CATH) was identified in cDNA from the venom gland of the Chinese cobra (*Naja atra*). A semi-conserved 11-residue pattern observed in the NA-CATH sequence provided the basis for generating an 11-residue truncated peptide, ATRA-1A, and its corresponding D-peptide isomer. While the antimicrobial and biophysical properties of the ATRA-1A stereoisomers have been investigated, their modes of action remain unclear. More broadly, mechanistic differences that can arise when investigating minimal antimicrobial units within larger naturally occurring CAMPs have not been rigorously explored. Therefore, the studies reported here are focused on this question and the interactions of full-length NA-CATH and the truncated ATRA-1A isomers with bacterial membranes. The results of these studies indicate that in engineering the ATRA-1A isomers, the associated change in peptide length and charge dramatically impacts not only their antimicrobial effectiveness, but also the mechanism of action they employ relative to that of the full-length parent peptide NA-CATH. These insights are relevant to future efforts to develop shorter versions of larger naturally occurring CAMPs for potential therapeutic applications.

1. Introduction

Cationic antimicrobial peptides (CAMPs) are pervasive in nature and represent an evolutionarily ancient mechanism for defending against invading microorganisms. These peptides exhibit broad spectrum antimicrobial effectiveness and are important elements of innate immunity, which provide the first line of defense against infection. Despite their widespread use, they exhibit limited bacterial resistance [1]. These qualities provide CAMPs an advantage over conventional therapeutics for fighting infections. Although CAMPs offer great potential as the basis for a new class of antibiotics, many of the details of the mechanisms by which they exert their antimicrobial effects remain unclear. Greater understanding of the relationship between CAMP physico-chemical properties and antimicrobial action is needed in order to realize their therapeutic potential.

CAMPs have been shown to interact with bacterial membranes and in many cases induce membrane disruption [2]. However, these interactions appear to be complex and the correlations between peptide physico-chemical properties, membrane composition and modes of action are poorly understood. CAMPs are usually amphipathic peptides presenting discrete cationic and hydrophobic surfaces. The spatial partitioning of these surfaces allows favorable electrostatic interaction with negatively charged lipid head groups on the outer surface of bacterial membranes and insertion into the hydrophobic interior of the bilayer, leading ultimately to membrane disruption [3,4]. Widely accepted membrane disruption mechanisms range from the "barrel-stave" model to the "carpet model". In the "barrel-stave" model, amphipathic helical peptides insert into the bacterial membrane, forming peptide lined structures with large central pores [2,5,6]. A similar proposed model is the "toroidal pore" where amphipathic helical peptides insert into the lipid membrane and form less defined transient supramolecular pores [5–7]. In the "carpet model," the peptides gather and concentrate at the membrane surface, interacting with the anionic lipid head groups, until the peptide concentration threshold is reached. This results in distortions in the lipid bilayer curvature and formation of transient gaps in the membrane [2,5,6].

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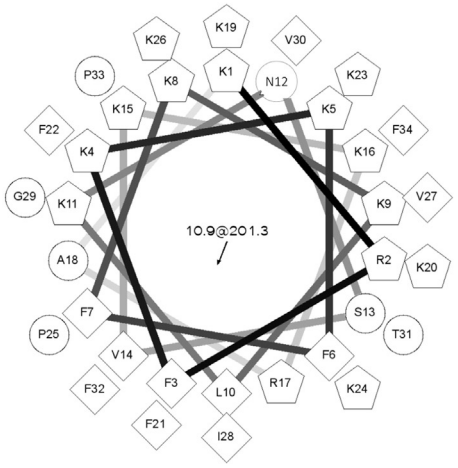
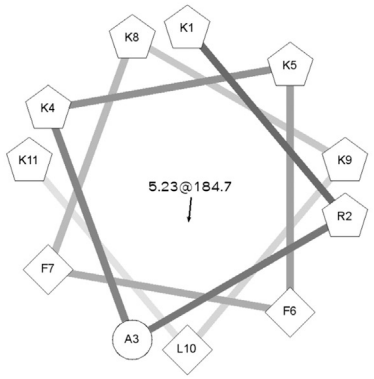
NA-CATH



Sequence	KRFKKFFKKLKNSVKKRAKK FFKKPKVIGVTFPF
Length (res)	34
Net Charge	+15

ATRA-1A



Sequence	KRAKKFFKKLK-NH_2
Length (res)	11
Net Charge	+ 8

Fig. 1. Helical wheel projection* and peptide sequences with associated net charge. The helical wheel projections of NA-CATH (left) and ATRA-1A (right) display hydrophilic residues as circles, hydrophobic residues as diamonds and positively charged residues as pentagons with the hydrophobic moment denoted in the center. Below the helical wheel projections are tables containing peptide sequence, length and charge information. The ATRA motifs are shaded gray in the NA-CATH sequence with the residues that differ between the motifs underlined. * Generated using http://rzlab.ucr.edu/scripts/wheel/wheel.cgi.

CAMPs can be grouped into families based on multiple factors, such as evolutionary relationships, conserved sequence patterns and structural elements. Cathelicidins are a sequence diverse family of vertebrate CAMPs that have been identified based on the highly conserved cathelin domain present in the precursor protein [8–11]. Recently, the sequence of a 34-residue helical cathelicidin, NA-CATH, was identified from cDNA derived from the venom glands of the elapid snake, *Naja atra* [12,13]. Analysis of the NA-CATH sequence revealed a semi-conserved 11-residue repeated sequence pattern. The 11-residue peptide amide, ATRA-1A, was designed based on this pattern (Fig. 1) [14]. Furthermore, the D-isomer of ATRA-1A was also investigated because D-peptide isomers have been shown to be more resistant to proteases than the corresponding L-peptides, which could enhance a peptide's therapeutic utility [15–17]. Significant disparities were observed in the antimicrobial effectiveness of the ATRA-1A stereoisomers [15]. Moreover, circular dichroism studies noted subtle dissimilarities in the structural properties of the peptide isomers in the presence of model anionic membranes, and differences in their abilities to disrupt model membranes and induce aggregation became evident in turbidity studies and fluorescence microscopy. While these studies focused on subtle differences in the properties of the ATRA-1A peptide isomers and their interactions with model membranes, they did not consider full-length NA-CATH, and how its properties may compare to those of the shorter ATRA-1A isomers.

The present study focuses on mechanistic differences that may arise when studying full-length CAMPs and antimicrobially active truncated versions of the peptide in order to identify minimal peptide units retaining antimicrobial activity and regions of the larger peptides responsible for their antimicrobial activity. We have investigated the full-length CAMP NA-CATH and the L- and D-isomers of the truncated peptide ATRA-1A in order to explore how altering CAMP length can affect antimicrobial activity and interaction with bacterial membranes. Specifically, we aim to more clearly elucidate similarities and differences in the membrane disruption mechanisms employed by full-length NA-CATH and the ATRA-1A isomers by focusing on key aspects of peptide-induced membrane disruption and antimicrobial kinetics in high and low salt environments against *Escherichia coli* and *Bacillus cereus*.

2. Material and methods

2.1. Materials

The peptides used in these studies were custom synthesized by AAPPTEC, LLC (Louisville, KY).[2] The supplier reported purities of NA-CATH, L-ATRA-1A and D-ATRA-1A were 95.0%, 95.2% and 95.4%, respectively, based on high-performance liquid chromatography (HPLC) analysis of the purified peptides. The bacterial strains of *Escherichia* (*E.*) *coli* (ATCC# 25922) and *Bacillus* (*B.*) *cereus* (ATCC# 11778) used in these studies were purchased from the American Type Culture Collection (Manassas, VA). 3,3′-dipropylthiacarbocyanine ($diSC_3$-(5)) was purchased from AnaSpec (Fremont, CA). SYTOX Green was purchased from Invitrogen (Carlsbad, CA). Mueller Hinton Broth (MHB) was purchased from Becton Dickinson and Company (Sparks, MD). 4-(2-Hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) was purchased from Acros Organics (New Jersey, US). Phosphate buffered saline (PBS) was purchased from Corning-cellgro (Manassas, VA). Resazurin, sodium salt was purchased from Sigma-Aldrich (St. Louis, MO). A SpectraMax Gemini EM was used for all experiments utilizing a plate-reading fluorimeter (Molecular Devices, Sunnyvale, CA).

2.2. Antimicrobial activity

The antimicrobial performances of NA-CATH, L-ATRA-1A and D-ATRA-1A were determined using a resazurin-based assay [18,19]. Frozen enumerated bacterial aliquots were thawed on ice and gently mixed.

[2] Commercial equipment and material suppliers are identified in this paper to adequately describe experimental procedures. This does not imply endorsement by NIST.

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