RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2015, **5**, 14214

Elucidating the mechanism of peptide interaction with membranes using the intrinsic fluorescence of tryptophan: perpendicular penetration of cecropin B-like peptides into *Pseudomonas aeruginosa*

Osama K. Abou-Zied,*[a] Abdelahhad Barbour,[b] Nada A. Al-Sharji[a] and Koshy Philip*[b]

The importance of small molecular weight antimicrobial peptides as novel therapeutic agents stems from their ability to act against bacteria, viruses, and fungi. As part of the innate immune system, they are also capable of killing cancerous cells. Herein, we study the interaction between a synthetic cecropin B peptide and a target *Pseudomonas aeruginosa* (PA) membrane using steady-state and time-resolved fluorescence measurements in order to elucidate the mechanism of membrane rupture. The importance of synthetic cecropin B as a therapeutic peptide stems from its effect against a wide range of bacteria which is indistinguishable from that of naturally occuring cecropins. Fluorescence of cecropin B results from the sole tryptophan residue in the peptide. In order to understand the mechansim of peptide–membrane binding, we modified the original peptide (cecropin B1: KWKVFKKIEKMGRNIRNGIV) by attaching a terminal tryptophan residue (cecropin B2: KWKVFKKIEKMGRNIRNGIVW). Both peptides show a large inhibition effect against a wide range of bacteria, compared to naturally occurring peptides. The fluorescence results show an enhancement in the peak intensity of cecropin B1 upon mixing with the membrane, accompanied by a blue shift. For cecropin B2, a blue shift was observed upon mixing with the PA membrane, but no enhancement in intensity was observed. The results indicate perpendicular penetration of cecropins B1 and B2 from the Lys side where the Trp residue of cecropin B1 is immersed in the PA membrane. Partial quenching of the Trp fluorescence by acrylamide was observed and the values of the Stern–Volmer constants (K_{sv}) indicate that the Trp molecule penetrates into the membrane, but resides close to the interface region. Two fluorescence lifetimes were measured for the cecropin B1–PA complex which are for two rotamers of Trp. The results point to a degree of flexibility of the local environment around the Trp molecule. A mechanism of membrane disruption is proposed in which the cecropin peptide creates cracks through the negatively charged outer membrane of PA.

Received 25th November 2014
Accepted 15th January 2015

DOI: 10.1039/c4ra15246h

www.rsc.org/advances

1 Introduction

Antimicrobial peptides (AMPs) are oligopeptides consisting of amino acid residues and can be found in both prokaryotes and eukaryotes.[1,2] To date, more than 5000 AMPs have been discovered and characterized.[3] Cecropins are lytic antimicrobial peptides originally isolated from the haemolymph of the *Hylophora cecropia*.[4] Cecropins were first isolated in 1980 and usually are of 35 to 37 residues length.[5] Cecropin B was found to be not only effective against a range of gram positive and gram negative bacteria but also cytotoxic to a range of mammalian cancer and non-cancer cell-lines.[6] Attempts to generate recombinant and chemically synthesized cecropin B were successful and the results indicate that naturally produced and synthetic cecropins are indistinguishable in terms of their antibacterial activity.[7] Cecropin B and its analogs showed to penetrate bacterial cell membranes and this permeabilization depends on the liposome composition in the targeted cells.[8] The structure of cecropin A has been studied previously by NMR spectroscopy and shown to consist of an amphiapathic α-helical N-terminus (which plays the main role in the antibacterial activity of cecropins), a glycine–proline bend and a hydrophobic C-terminal α-helix.[9] Unlike other amphiapathic α-helical peptides such as maganin[10] and dermaseptin,[11] cecropins do not lyse erythrocytes. The mode of action of cecropin is membrane permeabilization *via* peptide–lipid interaction rather than receptor-mediated recognition.[12]

In the present study, we investigate a model of peptide–membrane interaction using synthetic cecropin B-like peptides against the outer membrane of *Pseudomonas aeruginosa*. The

[a]*Department of Chemistry, Faculty of Science, Sultan Qaboos University, P.O. Box 36, Postal Code 123, Muscat, Sultanate of Oman. E-mail: abouzied@squ.edu.om; Fax: +968-2414-1469; Tel: +968-2414-1468*

[b]*Division of Microbiology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia. E-mail: kphil@um.edu.my; Fax: +60-3-7967-5908; Tel: +60-1-2395-1839*