Colloids and Surfaces A: Physicochem. Eng. Aspects 480 (2015) 307–317

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Membrane interaction of a new synthetic antimicrobial lipopeptide sp-85 with broad spectrum activity

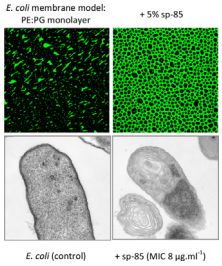
Ariadna Grau-Campistany[a,b], Montserrat Pujol[a], Ana M. Marqués[c], Ángeles Manresa[c], Francesc Rabanal[b], Yolanda Cajal[a,*]

[a] Department of Physical Chemistry, Faculty of Pharmacy, University of Barcelona, Joan XXIII s/n, 08028 Barcelona, Spain
[b] Department of Organic Chemistry, Faculty of Chemistry, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain
[c] Laboratory of Microbiology, Faculty of Pharmacy, University of Barcelona, Joan XXIII s/n, 08028 Barcelona, Spain

HIGHLIGHTS

- Sp-85 is a new synthetic antibiotic lipopeptide with broad spectrum activity.
- Pressure–area curves of mixed monolayers show non-ideal mixing.
- Binding to anionic liposomes results in fusion and leakage of aqueous contents.
- TEM images of antibiotic-exposed bacteria show damaged membranes.
- A mechanism of action based on bacterial membrane destabilization is proposed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 July 2014
Received in revised form 23 October 2014
Accepted 31 October 2014
Available online 8 November 2014

Keywords:
Antimicrobial peptide
Lipid–peptide interaction
Polymyxin B analogs
Model membrane
Langmuir monolayer
Liposomes

ABSTRACT

Antimicrobial peptides offer a new class of therapeutic agents to which bacteria may not be able to develop genetic resistance, since their main activity is in the lipid component of the bacterial cell membrane. We have developed a series of synthetic cationic cyclic lipopeptides based on natural polymyxin, and in this work we explore the interaction of sp-85, an analog that contains a C12 fatty acid at the N-terminus and two residues of arginine. This analog has been selected from its broad spectrum antibacterial activity in the micromolar range, and it has a disruptive action on the cytoplasmic membrane of bacteria, as demonstrated by TEM. In order to obtain information on the interaction of this analog with membrane lipids, we have obtained thermodynamic parameters from mixed monolayers prepared with POPG and POPE/POPG (molar ratio 6:4), as models of Gram positive and Gram negative bacteria, respectively. Langmuir–Blodgett films have been extracted on glass plates and observed by confocal microscopy, and images are consistent with a strong destabilizing effect on the membrane organization induced by sp-85. The effect of sp-85 on the membrane is confirmed with unilamelar lipid vesicles of the same composition, where biophysical experiments based on fluorescence are indicative of membrane fusion and permeabilization starting at very low concentrations of peptide and only if anionic lipids are present. Overall, results described here provide strong evidence that the mode of action of sp-85 is the alteration of the bacterial membrane permeability barrier.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

For the last decades antimicrobial resistance has been a growing threat to the effective treatment of an ever-increasing range

* Corresponding author. Tel.: +34 34024553.
E-mail address: ycajal@ub.edu (Y. Cajal).

http://dx.doi.org/10.1016/j.colsurfa.2014.10.062
0927-7757/© 2014 Elsevier B.V. All rights reserved.

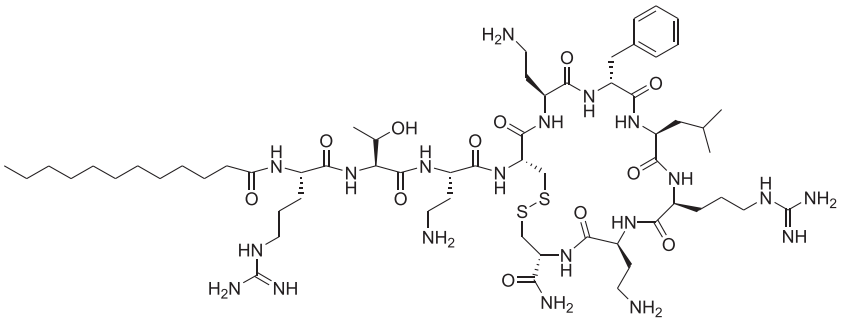


Fig. 1. Structure of sp-85, a synthetic decapeptide antibiotic formed by a (i) 7-member ring with 3 positive charges due to two 1,3-diaminobutyric acid (Dab) and one arginine (Arg) residues, and a hydrophobic segment (D-Phe-Leu), and (ii) a linear N-terminal region of 3 aminoacids with two positive charges (Dab and Arg), and a C12 fatty acid.

of bacterial infections, and it is now a complex public health challenge [1]. Some factors that have contributed to the development of resistance to current antibiotics include inappropriate use, such as the overuse of powerful, broad-spectrum antibiotics, the presence of antibiotics in the food/livestock industry, and the inclusion of antimicrobials in household products [2]. The World Health Organization recognizes antimicrobial resistance as one of the three greatest threats to human health [3]. However, the antimicrobial pipeline remains unacceptably lean, in fact, the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter sp*) have outpaced the drug discovery process [4,5]. This lack of new antimicrobials to replace those that become ineffective brings added urgency to the need to protect the efficacy of existing drugs. In the last few years there has been a resurgence of old antibiotics, and specially polymyxins discovered in the 1940s [6], as drugs of last resort for the treatment of infections caused by multi-drug resistant Gram-negative pathogens, despite their toxicity (nephro- and neurotoxicity) and the lack of clinical efficacy data [7,8]. Polymyxins belong to the class of antimicrobial peptides (AMPs), a class of antibiotics that have attracted great interest in the last few years because they rarely spur the development of resistant organisms. AMPs are a diverse group of molecules that share a few common features, the most important is that their main target is the lipid bilayer itself, and in consequence to develop genetic resistance is very costly for bacteria. AMPs are natural weapons produced by a variety of organisms, from single-celled microbes to vertebrates [9]. To date more than 2000 have been identified in nature and they are listed in the Antimicrobial Peptide Database (http://aps.unmc.edu/AP/main.php). They are very diverse in their sequences, length (generally short) and structures (some of them are linear while others are cyclic sometimes due to disulfide bridges, with a variety of secondary structures), but they adopt an amphipathic conformation that allows them to interact and disrupt selectively the negatively charged microbial membranes. No single mechanism can be defined for all AMPs, but despite these differences it can be concluded that they act mainly by binding to membranes and kill bacteria by disrupting membrane packing and organization, causing defects in the membrane with the consequent dissipation of transmembrane potential and leakage of important cellular contents [10–12]. In the case of PxB as well as cecropin and other AMPs, the proposed mechanism of action is based on the formation of periplasmic membrane contacts between outer and inner membranes. According to this model, once in the periplasmic space PxB forms contacts between the two enclosed phospholipid interfaces and promotes a fast exchange of certain phospholipids. The resulting changes in the membrane lipid composition trigger an osmotic imbalance that leads to bacterial stasis and cell death [13–15]. Sp-85 is a synthetic lipopetide that belongs to a family of more than 100 derivatives obtained by rational design based on the structure of natural antibiotic PxB [16–18]. The structure (Fig. 1) maintains the characteristics of PxB that are important for antibacterial activity, such as a cyclic nature, an overall positive charge, and an amphipathic structure, with two hydrophobic domains: a fatty acid in the N-terminus and two hydrophobic aminoacids in position 6–7 of the cycle. In this analog, some of the natural Dab residues of PxB have been substituted by Arg, another basic and positively charged aminoacid that is known to interact more strongly with the anionic membranes due to the particular properties of the guanidine group, providing arginine with strong bidentate cationic character and hydrogen-bond forming properties [19]. This allows arginine to form cation–π interactions that make insertion into the hydrophobic core of the bilayer energetically more favorable [20]. Herein we present biophysical studies to understand the membrane disruption mechanism for sp-85 using monolayers and bilayers as bacterial membrane models. Gram positive cytoplasmic membrane is modeled with POPG, whereas the Gram negative membrane is best mimicked with a binary mixture of POPE:POPG (molar ratio 6:4) [21]. Eukaryotic membranes tend to be neutral, and are modeled with zwitterionic POPC. Results are discussed in the light of the antibacterial activity of sp-85 on representative Gram positive and Gram negative bacteria and with its hemolytic activity *in vitro*.

2. Experimental

2.1. Chemicals

Phospholipids: 1-palmitoyl-2-oleoyl-glycero-*sn*- glycero-3-phospho-(1′-*rac*-glycerol) (POPG), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (DPPG), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were from Avanti Polar Lipids (Alabaster, Ala). Fluorescently labeled phospholipids and probes: 1-oleoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino] dodecanoyl]-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (NBD-PG), 1-hexadecanoyl-2-(1-pyrenedecanoyl) glycero-*sn*-3-phospho-(1′-*rac*-glycerol) (pyPG), 1-hexadecanoyl-2-(1-pyrenedecanoyl) glycero-*sn*-3-phosphocholine (pyPC), ANTS, 1-aminonapthalene-3,6,8-trisulfonic acid, and DPX, *N*,*N*′-*p*-xylenebis(pyridinium bromide) were purchased from Invitrogen Molecular Probes (Eugene, OR). *N*-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids were purchased from Bachem (Bubendorf, Switzerland) and Fluka (Buchs, Switzerland). Chemical reagents *N*,*N*-diisopropylcarbodiimide (DIPCDI), *N*-hydroxybenzotriazole (HOBt), trifluoroacetic acid (TFA) (BioChemika quality) as well as dodecanoic acid were also from Fluka (Buchs, Switzerland). Rink amide resin was purchased from Novabiochem (Läufelfingen, Switzerland). Chloroform and methanol (HPLC grade, Fisher Scientific CO) were used as the

Download English Version:

https://daneshyari.com/en/article/592166

Download Persian Version:

https://daneshyari.com/article/592166

Daneshyari.com