Biochimica et Biophysica Acta 1848 (2015) 3021–3025

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

S. Typhimurium strategies to resist killing by cationic antimicrobial peptides☆

Susana Matamouros [a], Samuel I. Miller [a,b,c,*]

[a] *Departments of Microbiology, University of Washington, Seattle, WA 98195, USA*
[b] *Departments of Genome Sciences, University of Washington, Seattle, WA 98195, USA*
[c] *Department of Medicine, University of Washington, Seattle, WA 98195, USA*

ARTICLE INFO

Article history:
Received 5 December 2014
Received in revised form 18 January 2015
Accepted 21 January 2015
Available online 30 January 2015

Keywords:
Salmonella Typhimurium
Resistance to cationic antimicrobial peptide
Polymyxin
Outer-membrane remodeling
Lipid A modification

ABSTRACT

S. Typhimurium is a broad host range Gram-negative pathogen that must evade killing by host innate immune systems to colonize, replicate, cause disease, and be transmitted to other hosts. A major pathogenic strategy of *Salmonellae* is entrance, survival, and replication within eukaryotic cell phagocytic vacuoles. These phagocytic vacuoles and gastrointestinal mucosal surfaces contain multiple cationic antimicrobial peptides (CAMPs) which control invading bacteria. *S.* Typhimurium possesses several key mechanisms to resist killing by CAMPs which involve sensing CAMPs and membrane damage to activate signaling cascades that result in remodeling of the bacterial envelope to reduce its overall negative charge with an increase in hydrophobicity to decrease binding and effectiveness of CAMPs. Moreover *Salmonellae* have additional mechanisms to resist killing by CAMPs including an outer membrane protease which targets cationic peptides at the surface, and specific efflux pumps which protect the inner membrane from damage. This article is part of a Special Issue entitled: Bacterial Resistance to Antimicrobial Peptides.

Contents

1. Introduction . . . 3021
2. Regulatory systems . . . 3022
3. Regulation of O-antigen length . . . 3022
4. Reduction of the anionic character of the bacterial surface . . . 3023
5. Decrease in bacterial surface membrane fluidity . . . 3023
6. Binding and eflux of CAMPs . . . 3024
7. Proteolytic degradation of CAMPs . . . 3024
8. Conclusion . . . 3024
Conflict of interest . . . 3024
Acknowledgments . . . 3024
References . . . 3024

1. Introduction

Antimicrobial peptides (AMPs) are ubiquitously produced as part of the mammalian innate immune systems and make up the entire immune system of insects and many non-vertebrate animals. They are structurally diverse small amphipathic peptides in which a cationic surface and a hydrophobic surface facilitate interaction with biological membranes [1–4]. Unlike eukaryotic cells, the bacterial outer layer net surface charge is anionic [2]. Gram-negative bacteria have negatively-charged lipopolysaccharides (LPS) as the major constituent of the outer-leaflet of their outer-membranes and Gram-positive bacteria have acidic polysaccharides (teichoic and teichuronic acids) decorating their surface. Consequently most AMPs with activity against bacteria are cationic which allows binding to the negatively charged bacterial surface [2–4]. Thus, surface remodeling aimed at decreasing the negative charge

Abbreviations: AMP, antimicrobial peptides; CAMP, cationic antimicrobial peptides; LPS, lipopolysaccharide; CRAMP, cathelicidin-related anti-microbial peptide

☆ This article is part of a Special Issue entitled: Bacterial Resistance to Antimicrobial Peptides.

* Corresponding author at: Department of Microbiology, University of Washington, Seattle, WA 98195, USA.
E-mail address: millersi@uw.edu (S.I. Miller).

http://dx.doi.org/10.1016/j.bbamem.2015.01.013
0005-2736/

and increasing hydrophobicity of bacterial membranes is often employed as a first line of defense in bacterial resistance to antimicrobial peptides. Remodeling of the bacterial surface has been extensively studied in the Gram-negative pathogenic bacterium *Salmonella enterica* serovar Typhimurium. *Salmonella* is able to reduce recognition by the host innate immune system and binding of CAMPs to its surface by specific modification of its outer membrane components. Several strategies have been identified in *Salmonellae* and other Gram-negative bacteria that result in the modification of the LPS structure and properties as well as sequestering, efflux and proteolytic degradation of AMPs.

2. Regulatory systems

Salmonella possesses several regulatory systems that control modifications necessary for AMP resistance. The regulation of the proteins involved in these processes is complex and involves several regulatory proteins such as the alternative sigma factor, RpoE [5] and several two-component systems: PhoPQ, PmrAB and RcsABCDF (Fig. 1) [6–8]. *S.* Typhimurium and other Gram-negative bacteria regulate most changes to the outer-membrane through the two-component system PhoPQ [9–11]. PhoQ is an inner-membrane histidine kinase that is activated once *Salmonella* is inside host cells and responds to low pH and the presence of AMPs [7,12–14]. The presence of these signals leads to the phosphorylation of the cytoplasmic response regulator PhoP (Fig. 1). PhoQ periplasmic domain contains a negatively charged surface (acidic patch) facing the membrane that is tethered to the anionic phospholipid head-groups of the inner-membrane by the presence of metal ions such as Ca^{2+} and Mg^{2+} keeping it in a repressed state [15]. Direct interaction of the PhoQ periplasmic domain acidic patch with the positively charged surface of CAMPs disrupts the metal bridges and induces conformational changes in PhoQ that lead to PhoQ autophosphorylation and subsequent transfer of the phosphate group to PhoP (Fig. 1) [13]. Once phosphorylated, PhoP controls the expression of several genes involved in resistance to AMPs and intracellular survival and hence virulence.

PhoPQ also regulates the transcription of another two-component system, PmrAB that is necessary for *Salmonella* resistance to AMPs [6,16]. Upon activation PhoP induced synthesis of PmrD prevents the dephosphorylation of PmrA keeping it in an activating state (Fig. 1) [17,18]. Activation of PmrAB can also occur in the presence of ferric iron or low pH [19,20]. PmrA directly controls the expression of several genes involved in LPS modifications that are absolutely necessary for resistance to Polymyxin B and other CAMPs.

The Rcs regulatory system involves several regulatory proteins and a multiple-step phosphotransfer cascade. It is composed of two inner-membrane sensors, the RcsC hybrid kinase and the RcsD histidine phosphotransfer protein, an outer membrane inner leaflet and periplasmic lipoprotein RcsF and two cytoplasmic regulatory proteins, RcsA and RcsB (Fig. 1) [21,22]. RcsF is essential for the activation of this system in the presence of CAMP, likely through specific disorder of the outer membrane and alteration in the localization or conformation of RcsF [8]. The Rcs system controls the expression of the capsular polysaccharide genes as well as other genes necessary for *Salmonella* resistance to antimicrobial peptides and persistence in mice [23,24].

3. Regulation of O-antigen length

The outer-leaflet of the outer-membrane of Gram-negative bacteria is mainly composed of LPS. LPS is a complex amphiphilic molecule composed of three distinct domains: the hypervariable and highly immunogenic O-region formed by repeating oligosaccharide subunits; a short core oligosaccharide region usually common to all members of a bacterial genus and; a membrane anchoring hydrophobic lipid composed of a phosphorylated glucosamine disaccharide that in enteric bacteria most often has 6 attached fatty acids, termed lipid A. Since LPS is the major component of the outer leaflet of the outer membrane of Gram-negative bacteria, it is therefore a first barrier in the defense against external toxins such as AMPs. Being the outermost portion of the LPS molecule and bacterial cellular surface, the O-antigen serves as a protective barrier from CAMPs and other membrane active compounds. It hides the negatively charged phosphate groups of the core and lipid A inhibiting the electrostatic attraction between the CAMPs and these two domains of the LPS molecule. *S.* Typhimurium can produce short LPS species with the O-antigen domain containing between 1 and 15 oligosaccharide repeat units, long LPS species that have 16 to 35 O-antigen repeat units [25], or very long LPS chains can have over 100 repeat

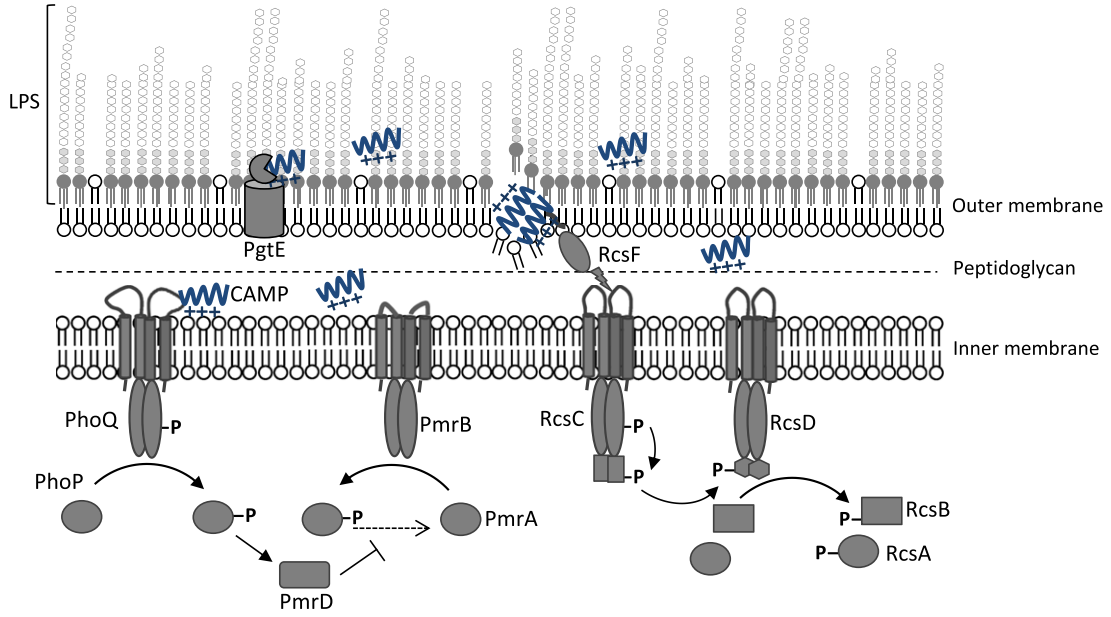

Fig. 1. Regulatory systems that control several modifications essential for *Salmonella* resistance to CAMPs. Upon exposure to CAMPs there is disruption of the outer membrane barrier. PhoQ is able to directly bind CAMPs via its periplasmic domain which results in activation of the PhoPQ and the PmrAB operons. These operons encode several proteins essential for resistance to CAMPs, among which is the outer-membrane protease PgtE. Activation of the Rcs phosphorelay system occurs via the outer-membrane lipoprotein RcsF that senses membrane damage caused by CAMPs. The concerted activation of these regulons results in increased resistance to CAMPs.