Biochimica et Biophysica Acta 1794 (2009) 808–816

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Outer membrane permeability and antibiotic resistance

Anne H. Delcour *

Department of Biology and Biochemistry, University of Houston, 369 Science and Research Building II, Houston, TX 77204-5001, USA

ARTICLE INFO

Article history:
Received 19 September 2008
Received in revised form 12 November 2008
Accepted 13 November 2008
Available online 27 November 2008

Keywords:
Outer membrane
Antibiotic
Porin
LPS
Resistance

ABSTRACT

To date most antibiotics are targeted at intracellular processes, and must be able to penetrate the bacterial cell envelope. In particular, the outer membrane of gram-negative bacteria provides a formidable barrier that must be overcome. There are essentially two pathways that antibiotics can take through the outer membrane: a lipid-mediated pathway for hydrophobic antibiotics, and general diffusion porins for hydrophilic antibiotics. The lipid and protein compositions of the outer membrane have a strong impact on the sensitivity of bacteria to many types of antibiotics, and drug resistance involving modifications of these macromolecules is common. This review will describe the molecular mechanisms for permeation of antibiotics through the outer membrane, and the strategies that bacteria have deployed to resist antibiotics by modifications of these pathways.

The outer membrane (OM) of gram-negative bacteria performs the crucial role of providing an extra layer of protection to the organism without compromising the exchange of material required for sustaining life. In this dual capacity, the OM emerges as a sophisticated macromolecular assembly, whose complexity has been unraveled only in recent years. By combining a highly hydrophobic lipid bilayer with pore-forming proteins of specific size-exclusion properties, the OM acts as a selective barrier. The permeability properties of this barrier, therefore, have a major impact on the susceptibility of the microorganism to antibiotics, which, to date, are essentially targeted at intracellular processes. Small hydrophilic drugs, such as β-lactams, use the pore-forming porins to gain access to the cell interior, while macrolides and other hydrophobic drugs diffuse across the lipid bilayer. The existence of drug-resistant strains in a large number of bacterial species due to modifications in the lipid or protein composition of the OM indeed highlights the importance of the OM barrier in antibiotic sensitivity. This review will summarize the properties of the OM lipid barrier and porin-mediated permeability, and highlight the antibiotic resistance mechanisms that involve modifications of these properties.

It is important to note that many of the alterations in outer membrane permeability described below are often associated with increased levels of antibiotic efflux. Even intrinsic antibiotic resistance is likely to reflect the synergistic action of the outer membrane acting as a permeability barrier, and of the diverse and widely distributed efflux pumps. The review below essentially focuses on the permeability changes per se, as the roles of efflux pathways in antibiotic resistance are treated by others. Whether changes in outer membrane lipid or porin composition also mechanistically influences the efflux systems remains to be determined.

1. Organization of the OM

In most gram-negative bacteria, the OM is an asymmetric bilayer of phospholipid and lipopolysaccharides (LPS), the latter exclusively found in the outer leaflet. A typical LPS molecule consists of three parts (Fig. 1): 1) lipid A, a glucosamine-based phospholipid, 2) a relatively short core oligosaccharide, and 3) a distal polysaccharide (O-antigen) [1]. Since part of the core oligosaccharide and the O-antigen are not required for the growth of *Escherichia coli*, strains can exhibit varying length of these structures. The phospholipid composition of the inner leaflet of the OM is similar to that of the cytoplasmic membrane, i.e. about 80% phosphatidylethanolamine, 15% phosphatidylglycerol and 5% cardiolipin [2]. In mutants with altered LPS structure, phospholipids have also been detected in the outer leaflet of the OM, possibly due to consequent decrease in OM protein levels [3].

A large number of different types of proteins reside in the OM. Some of them are extremely abundant. For example, murein lipoprotein (Lpp), OmpA and general diffusion porins are present at $>10^5$ copies per cell [4]. Lpp carries a fatty acid moiety that anchors it into the OM, while about a third of the Lpp population is also covalently attached to the peptidoglycan layer. Thus, Lpp is thought to play a role in providing OM–peptidoglycan interactions and in maintaining OM integrity. Indeed, mutants lacking Lpp produce OM vesicles and leak periplasmic enzymes [5]. Another abundant OM protein is OmpA. The protein is believed to have a structural role and the absence of OmpA and Lpp compromises the shape of the cell [6]. Along with the *Pseudomonas aeruginosa* homolog OprF, OmpA has pore-forming properties as well, but with extremely low permeation

* Tel.: +1 713 7432684; fax: +1 713743 2636.
E-mail address: adelcour@uh.edu.

1570-9639/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2008.11.005

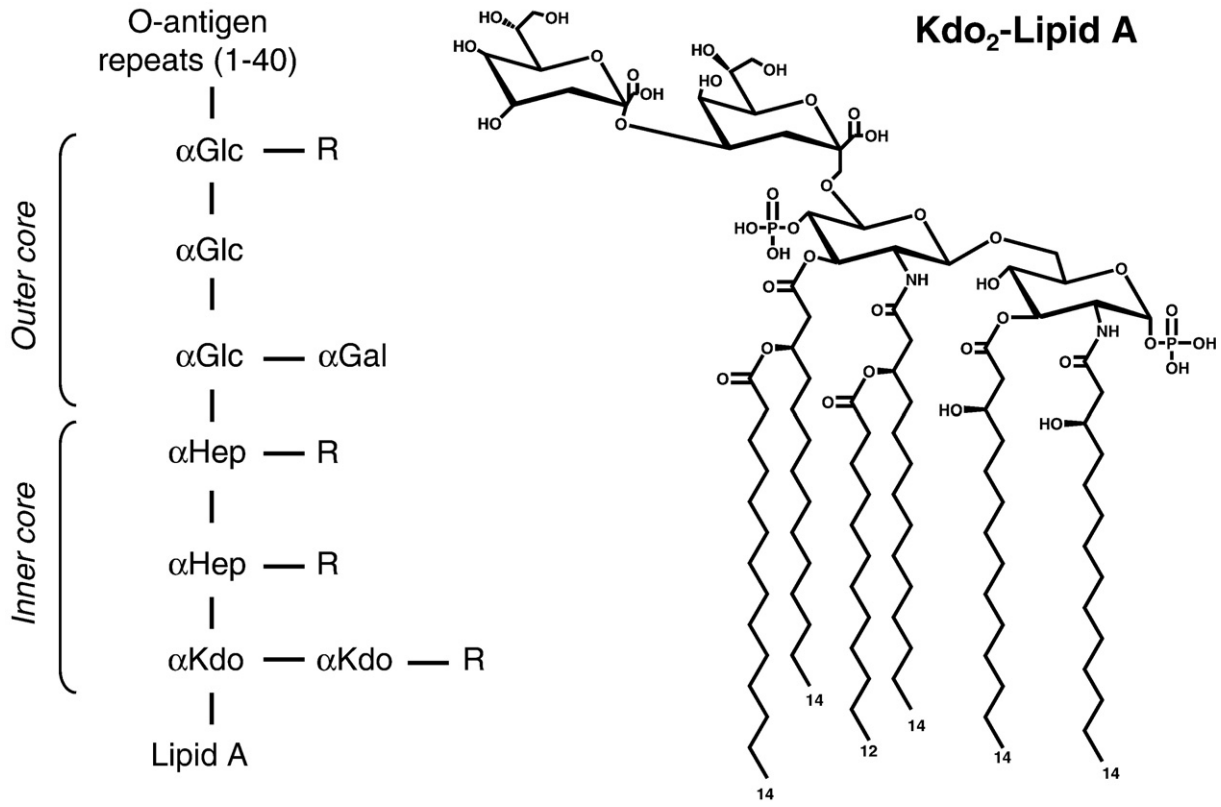

Fig. 1. Overall organization of LPS and structure of Kdo_2-Lipid A. The left hand side shows the organization of LPS in 3 regions: Lipid A, core oligosaccharide (itself subdivided into inner core and outer core), and O-antigen. Abbreviations are: Kdo, 3-deoxy-D-*manno*-oct-2-ulosonic acid; Hep, L-*glycero*-D-*manno*-heptose; Glc, D-glucose; Gal, D-galactose; R, a variety of different substituents (see reference [13] for details). The right hand side shows the structure of Kdo_2-Lipid A, the minimal entity required for *E. coli* growth.

efficiency. Recent experimental evidence suggests that these proteins exhibit two different conformations, an abundant closed form that exists as a monomeric 8-stranded β-barrel with a C-terminal periplasmic domain, and a rare oligomeric form, that comprises large open β-barrels similar to the general diffusion porin OmpF [7,8].

Other than general diffusion porins, which will be described in detail below, the OM also contains specialized protein channels and receptors used for the uptake of specific substrates (for example LamB and BtuB for maltodextrins and vitamin B12 transport, respectively), proteins involved in OM and surface appendages biogenesis (for example, Omp85 for membrane protein insertion, and a large array of translocators used in the assembly of adhesins, pili and flagella), translocons allowing release of secreted substrates (for example, translocon of the Type II secretion system involved in toxin release), various enzymes (such as the *E. coli* OmpT protease) and proteins involved in LPS assembly. The reader is referred to recent reviews for more information on these proteins [9–12].

2. The OM lipid barrier

2.1. Molecular description

The asymmetric presence of LPS is a salient and unique feature of the OM. LPS is composed of the hydrophobic, fatty acid chain bearing lipid A, a core oligosaccharide and the O-antigen (Fig. 1). The O antigen is an immunogenic oligosaccharide of considerable variability among gram-negative bacteria, consisting of 1 to 40 repeating units. The core oligosaccharide is branched and contains 6 to 10 sugars in addition to two Kdos (3-deoxy-D-*manno*-oct-2-ulosonic acid) linked to lipid A. This core region is also heterogeneous due to the variable presence and nature of additional substituents. Lipid A is a glucosamine disaccharide, phosphorylated at the 1 and 4′ positions, and acetylated at the 2, 2′, 3 and 3′ positions with 3-hydroxymyristic acid. It differs from a typical phospholipid by having six saturated fatty acid chains rather than two saturated or unsaturated chains. These characteristics make the asymmetric OM bilayer much more hydrophobic than a typical phospholipid bilayer, due to strong lateral interactions between LPS molecules and low fluidity [4]. The glucosamine backbone of lipid A and the core region bear multiple anionic groups, and LPS is known to bind strongly divalent cations, which compensates for the electrostatic repulsion between neighboring LPS molecules. Only the inner part of LPS, consisting of lipid A and Kdo, is required to sustain growth in *E. coli* [1]. Thus, many mutants (R or "rough" mutants, due to colony appearance) exist with varying length of core oligosaccharide, and have been classified as Ra to Re chemotypes [4,13]. "Deep rough" mutants have the most truncated core, and show high sensitivity to lipophylic agents such as detergents, some antibiotics, bile salts, etc. "Smooth" strains have an intact O-antigen, of varying length, and are found among clinical isolates of *Enterobacteriaceae*. Excellent descriptions of LPS structure and biogenesis can be found in earlier reviews [1,13].

2.2. Lipid-mediated antibiotic resistance

Hydrophobic antibiotics that appear to gain access to the cell interior by permeating through the OM bilayer *per se* are aminoglycosides (gentamycin, kanamycin), macrolides (erythromycin), rifamycins, novobiocin, fusidic acid and cationic peptides [11,14]. Tetracylcine and quinolones use both a lipid-mediated and a porin-mediated pathway (see below). The core region of LPS plays a major role in providing a barrier to hydrophobic antibiotics and other compounds, and the strains which express full length LPS have an intrinsic resistance to these. On the other hand, membrane permeabilizers, such as Tris/EDTA, polymyxin B and polymyxin B nonapeptide (PMBN), have the ability to increase the sensitivity of *E. coli* and *Salmonella typhimurium* to the hydrophobic antibiotics mentioned above