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Cell Envelope Structure and Functions in Gram-negative Bacteria

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The application of high resolution electron microscopy revealed fundamental differences between Gram-positive and Gram-negative bacteria. The cytoplasmic membrane of Grampositives is enclosed in a thick layer of poptidoglycan and in some cases covered by a capsule whereas the structure of the cell envelope in Gram-negative organisms is more complicated, and consists of cytoplasmic membrane and a complex cell wall, as shown in Figure 1.

I. Cytoplasmic membrane:

The first layer which limits the cellular protoplasm is the cytoplasmic or plasma membrane. The structure of this selectively permeable membrane is generally the same in both Gram-positive and Gram-negative bacteria. The most accepted model for such a cell membrane is the fluid mosaic model suggested by Singer and Nicolson (1972) and based on the unit membrane concept. They proposed that the membrane consists of a double layer of phospholipid moleculed with their hydrophobic fatty acid chains (hydrocarbon) pointing

inward and the hydrophilic phosphate groups facing outward (Robertson 1981). Moreover, two types of protein molecules have been recognized in this membbrane which lie within the two layers or on the surface of one lipid layer and somtimes extended across the width of the membrane (Fig. 2). One type of these proteins is more peripheral and can be easily isolated by some chelating

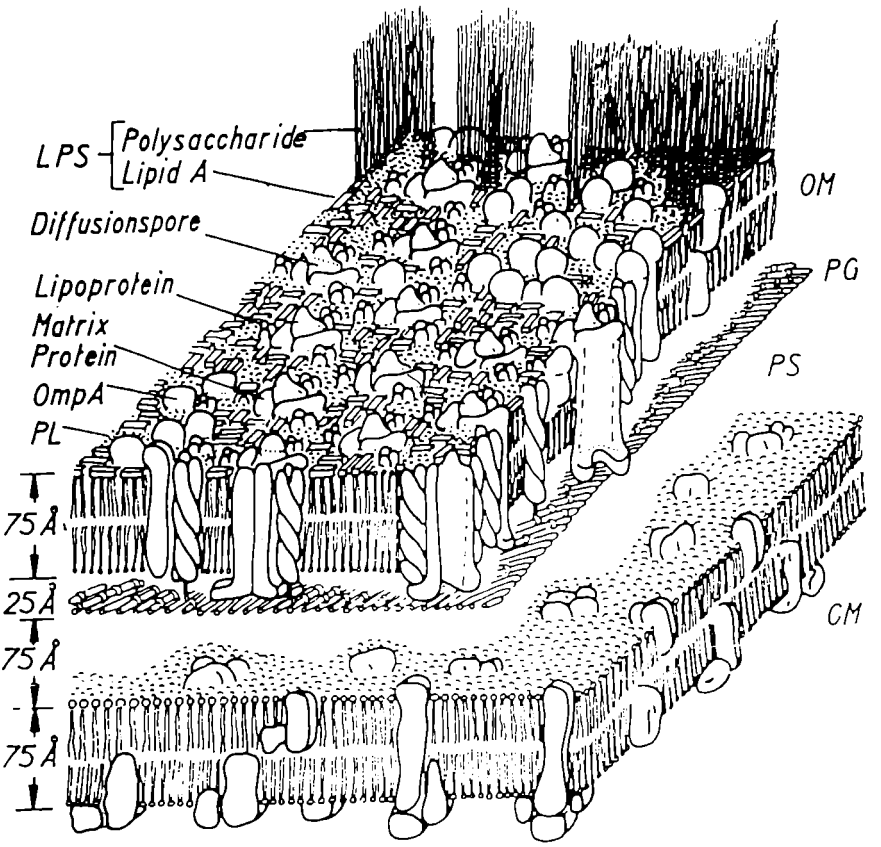

Figure 1. *Model of the Gram-negative cell envelope.*

LPS: Lipopolysaccharide; OM: Outer membrane; PG: Peptidoglycan PS: Periplasmic space; CM: Cytoplasmic membrane; OmpA: Outer membrane protein A; PL: Phospholipids.

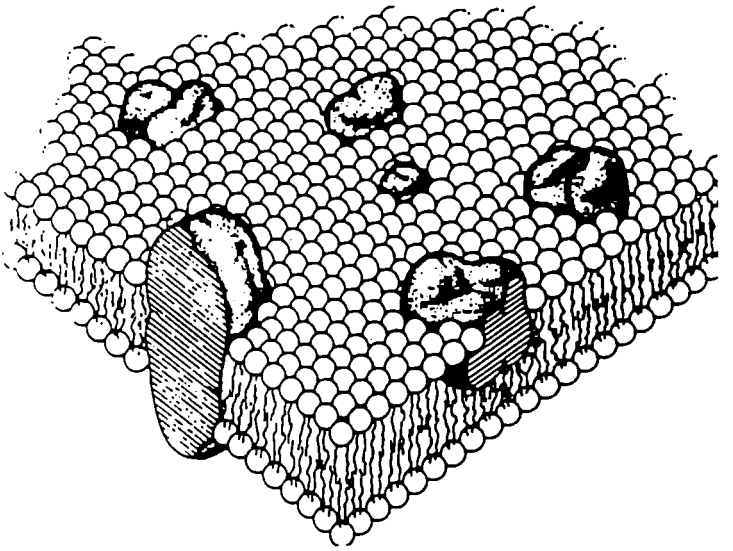

Figure 2. *Fluid mosaic model of cytoplasmic membrane from singer and Nocholson (1972). The empty circles represent the phosphate group with the hydrophobic tails. Proteins are designated by the cross lined particles embeded in the bilayer.*

agents such as EDTA, whereas the other types which are referred to as intrinsic ones are firmly bound to hydrophobic tails and can only be removed by dissociation of the cytoplasmic membrane (Rogers 1983). It is believed that these proteins are bound to phospholipid molecules by divalent cations (eg. Mg^{2+}). The cytoplasmic membrane controls the passage of metabolites in and waste products out and may also selectivly act as a barrier against lethal substances such as toxins and antibiotics. It is also a site for the biosynthesis of extracellular enzymes and toxins as well as for the electron transport chains (Hammond et al 1984). The lipid part regulates the permeability of the cell and provides the lipophilic environment required for membrane enzymes involved in the synthesis of lipopolysaccharides, peptidoglycan and in Gram-positives teichoic acid (Rogers et al 1980).

II. cell wall:

The cytoplasmic membrane is surrounded by a structured and mechanically strong layer called cell wall which is responsible for the shape maintenance and integrity of the bacterial cell. The cell wall is formed of the peptidoglycan layer, periplasmic region and the outer membrane.

II.1 Peptidoglycan:

The first layer of the cell wall which encases the plasma membrane, is the peptidoglycan or murein layer. This rigid layer in Gram-negative bacteria is very thin and constitutes only 5-10% of the cell wall dry weight, whereas, in Gram-poitives it is thick (50-60% of the wall dry weight) and is the only structure which covers the cytoplasmic memberane (Burge et al. 1977). Its stable complex with crystal violet in Gram staining is an important factor to differentiate between these two main bacterial groups. The basic structure of peptidoglycan layer is made of a network of polyaminosugar chains cross linked by short peptide molecules as shown in Figure 3. The polysaccharide chains or glycan strands sometimes consist of up to 200 disaccharide units which are alternatingly fromed by N-acetylmuramic acid and N-acetylglucosmine molecules (Iterson 1984). The peptedoglycan layer plays a crucial role in physical and physiological maintenance of bacterial cell particularly in Gram-positive organisms. Some antibiotics such as ß-lactams are effective on this layer by damaging the peptide cross-link of the peptidoglycan molecules.

II.2 Periplasmic region:

In Gram-negative bacteria the region between the cytoplasmic and the outer membrane is known as periplasmic region in which the peptidoglycan layer is situated and possibly forms a sponge-like gel in it (Hammond et al 1984). The periplasmic region also contains the aqueous periplasm, a solution of water soluble enzymes and unique series of other proteins and oligosaccharides. The impermeability of the outer membrane is the main factor inhibiting the leakage of periplamic contents to the medium. Some of the enzymes present in the periplasmic region are responsible for inactivation of the antibiotics which have been able to penetrate the permeability barrier (Hammond et al 1984). Certain other proteins in this region convert the nutritional compounds to a form which can be transferred through the cytoplasmic membrane.

II.3 Outer membrane:

The presence of the outer membrane in Gram-negative bacteria is physiologically very important for the bacterial cell. In comparison to Gram-positive organisms, the high level of resistance to antibiotics and toxins in Gram-negatives is due to the impermeability of the outer membrane to these compounds. In other words, this membrane constitutes a barrier protecting the bacteria against hydrophobic compounds (eg. antibiotics) as well as large hydrophilic macromolecules such as enzymes and toxins (Hammond et al. 1984).

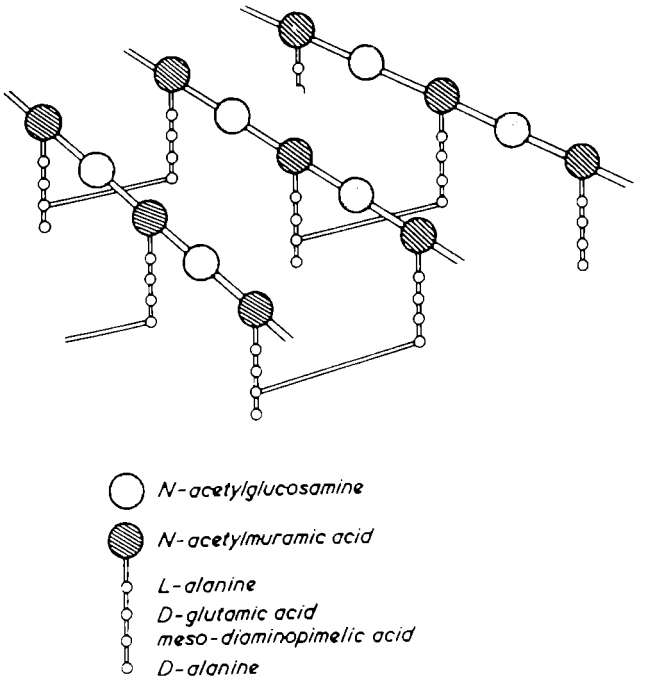

Figure 3. *The schematic structure of the peptidoglycan layer of the cell wall.*

The outer membrane consists of three major components, lipopolysaccharides, phospholipids and proteins. According to the accepted model of Singer-Nicholson the location of these molecules are shown in Figure 1. Like cytoplasmic membrane it is made of a bilayer sturcture of lipids with hydrophobic lipid chain inwards and hydrophilic groups outwards. The difference from the cytoplasmic membrane is that the outer membrane is not a phospholipid bilayer but it contains phospholipids and low molecular weight lipoproteins in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet.

II.3.a Lipopolysaccharides.

The main physiologically important structure of the outer membrane is formed by lipopolysaccharide molecules which are located in the exterior layer of the membrane. They are mainly associated with antigenic activity, the endotoxic properties of the bacterial cell and the outer membrane barrier properties.

The basic model of LPS molecules which has been suggested by Luderitz and Westphal (Luderitz et al 1982) consist of three regions; the lipid A region,

core polysaccharide and the O-side (O-specific) chain (Fig. 4). The two last parts are sometimes referred to as heteropolysaccharide which are covalently linked to the lipid A. There are two major differenes between the lipid structures of LPS and phospholipid molecules; the fatty acids of LPS are always saturated whereas those of the phospholipids are not. Also there are two lipid chains in phospholipids but the LPS molecules usually have up to seven fatty acid chains (Nilaido and Varra 1985). The LPS molecules are also strongly bound to divalent cations such as Mg^{2+} which gives them a firm position in the outer membrane (Schindler and Osborn 1979).

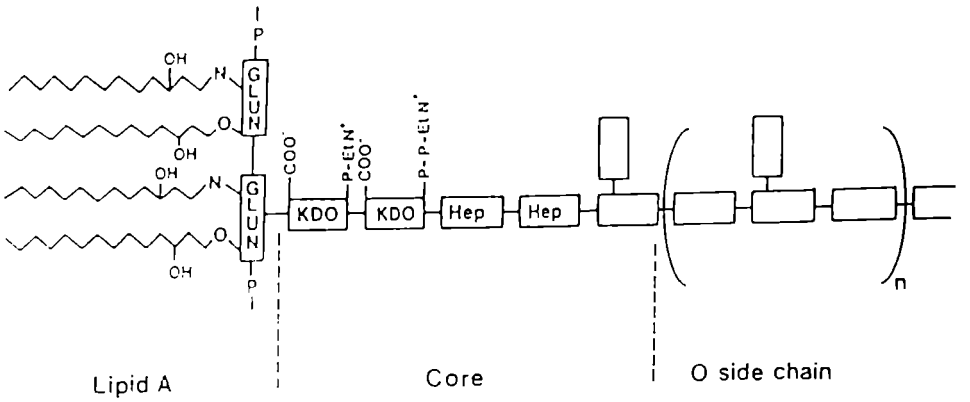

Figure 4. *Model of Lipopolysaccharide molecules of the outer membrane.*

The lipid A is a glycolipid structure embbedded in the membrane and is in fact the bacterial endotoxin. It is the only bacterial lipid which contains hydroxy fatty acid, mainly D-3-hydroxy acids (Hammond et al 1984). A diversity of lipid chains and D-3-hydroxy fatty acids may be found in Gram-negative bacteria. In enterobacteria these fatty acids are usually linked to glucosmine disaccharide; mainly ß-hydroxymyristate (Luderitz et al 1982).

The core polysaccharide is located outwards between lipid A and O-specific chain. it is made mainly from, glucose, galactose, glucosamine, mannoheptose, KDO and some phosphate residues. The sugars of the core are

linked to lipid A by the suger of the core are linked to lipid A by the suger acid 2-keto-3-deoxyoctonic acid (KDO) or 3-deoxy-D-mano-octulosonic acid (Nikaido and Varra 1985). Although many mutations resulting in the production of defective LPS molecules have been reported (Makela and Stocker 1981), the KDO region seems to be stable and mutation does not affect it (Hammond et al 1984). The core structure is similar in closely related bacterial strains.

The O-side chain extends freely outwards from the core region up to 30 nm. It has a basic structure consisting of a hydrophilic chain of repeating identical oligosaccharide subunits and may be either linear or branched. These are immunologically important structures since they determine the nature of cell surface antigens. The presence of a diverse range of O-serotypes in Gram-negative bacteria (up to one hundred in Salmonella and E. coli) is due to the variatiion in sugar composition and the type of glycoside linkage (Luderitz et al 1982). The synthesis of these polysaccharides are catalyzed by the enzymes associated with the cytoplasmic membrane.

The proteins in the outer membrane are of three types:

II.3.b Lipoproteins:

Which often extend into the periplasmic space and some of these lipoproteins are covalantly bound to peptidoglycan layer and the others are free in the periplasmic region. These are the most abundant peroteins in the bacterial cell and as small in molecular weight as 7200 daltons (Nikaido and Vaara 1985). For example their number in E.coli envelope has been reported to be around 7.5 x 10^5 per cell. As Mentioned they apear in two forms; about one-third of them are covalently linked to peptidoglycan layer by the amine group of lysine. The rest which are not bound to peptidoglycan contain free lysine. Lipoproteins can be detached from peptidoglycan by enzymatic treatment. Compared to the other outer membeane proteins the function of these proteins is not clearly understood. It is suggested taht lipoproteins particularly peptidoglycan-bound ones assist the stability of the outer membrane (Nikido and Vaara 1985), and mutants lacking these proteins were seen to have unstable membrane and were releasing the periplasmic contents into the environment (Hirota et al 1977).

II.3. C- Major proteins:

Are a group of proteins always present in the outer memtrane and can be readily detected by SDS polyacrylamide gel eletrophoresis (PAGE). They may act as binding sites for bacteriophages, bacteriocins and leucocytes or as diffusion pores for hydrophilic molecutes which may be used for entry of antibiotics. Certain bacteria demonstrating resistance to antibiotics or which do not bind leucocytes and phages, often possess modeified major proteins. The molecular

weight of these proteins ranges between 35000 to 38000 in E. coli K12 (Hammond et al 1984).

Based on the extraction conditions, two groups of major proteins have been indetified; those which can be extracted in 2% SDS at 60° such as OmpA (outer membrane protein a) and the other group which are not detectable under these conditions or require higher temperature like matrix proteins or porins.

II.3. C-1- OmpA:

Is a major protein readily extracted from the outer membrane in E. Coli. Also its molecular weight changes apparently with increased temperature when detected by SDS polyacrylamide gel electrophoresis. Thus when the preparation in SDS is heated its mobility reduces significantly, and therefore it is referred to as "heat modifiable" protein (Nakamura and Mizushima 1977). The exact function of OmpA is not precisely understood, but this protein acts as phage receptor (Lugtenberg and van Plpen 1983). It is reported that in E. coli the OmpA is the receptor of phage K3 and Tu II (Hammond et al 1984). Some mutants of E. coli lacking this protein exhibited defective growth rate due to the reduced amino acid transport system (Manning et al 1977).

II.3. C-2- Porin protein:

Is a type of outer membrane major protein which can be detected at high temperature in SDS-PAGE. This is a trimer channelled molecule of protein which lies across the outer membrance and is linked to peptidoglycan layer. The channels formed by porins allow the non-specific diffusion of small hydrophilic molecules (less than 700) of nutrients, antibiotics and other inhibitors to cross the membrane (Lutkenhaus 1977). These proteins also serve as receptors for bacteriophages and bacteriocins. Recent electron microscopy studies showed that each of the three subunits of porins have a separate channel which in the middle of their length join to each other and form a single channel at the other side of the outer membrane (Dorset et al 1984). These proteins are coded for by *ompC, ompF* and *phoE* determinants in E. coli K12 (Nikaido and Vaara 1985). (Nurminen et al 1976) reported the presence of another type of porin in an OmpD mutant of *Salmonella typhimurium*. Also *E. coli* mutants lacking *OmpC* and *OmpF* proteins which were resistant to specific phages and bacteriocins have been observed (Hammond et al 1984).

II.3. D- Minor proteins:

Which are inducible or dereppressible proteins and may be induced or altered by genetic determinants such as, plasmids and phages also under specific

environmental or nutritional circumstances. There are different types of these proteins. Most of the specific diffusion channels are in this class. LamB protein in *E. coli* K12 is a minor protein which is specifically a channel for the passage of maltose and maltodextrins (Nikaido and VAara 1985). The Tsx protein is a constitutive minor protein with 26000 molecular weight which acts as a receptor for phage t6 and is involved in nucleoside transport.

II.3. E- Phospholipids:

The phospholipids of the outer membrance are almost the same as those of the cytoplasmic membrane, but most of the outer membrane phospholipids are of phosphatidylethanolamine PE and less phosphtidylglycerol PG (Lugtenberg and Peters 1976). Also the ratio of saturated fatty acids particularly in PE is significantly higher than unsaturated ones. This composition is not affected by the conditions of the growth medium. The phospholipid molecules of the outer membrane are predominantly present in the inner leaflet of the membrane (Hammond et al 1984). It is suggested that phospholipids are involved in the permeability barrier of the outer membrane, and the mutants with defective phospholipids were more sensitive to antibiotics (Lugtenberg and Peters 1976).

II.3. F- Properties of the outer membrane:

As metioned earlier the presence of the cell wall is of great biological importance to Gram-negative bacteria which is discussed below.

A. Resistance to enzymes.

The outer membrane renders the host organism resistant to body defence systems like lysozyme and antibodies. For example many body fluids such as tears and serum contain the enzyme lysozyme which has a significant role in the host defence system. In Gram-positive bacteria this enzyme easily degrades and removes the poptidoglycan layer of the cell followed by lysis (Patterson-Delafield et al 1980). In Gram-negatives, however due to the presence of the outer membrane this enzyme is ineffective on peptidoglycan. Lysozyme can only be active on this group of bacteria if the outer membrane is removed by a chelating agent like EDTA. Moreover, the outer membrane is the main protective structure in enteric bacteria living as normal inhabitants of intestinal tract, against the digestive enzymes and bile salts (Nikaido and Nakae 1977). This protective role is mainly due to the permeability barrier of the outer membrane. As metioned above, hydrophilic molecules with molecular weight more than 700 can not pass through the porin proteins. Also the strong interaction between the LPS molecules and the segregation of these molecules and phospholipids plays

and essential role in survival of bacteria against the lytic effect of enzymes and detergents (Nikaido and Vaara 1985).

B. Resistance to hydrophobic molecules:

The outer membrane of Gram-negative bacteria forms an impermeable barrier against the hydrophobic molecules such as erythromycin, novobiocin, rifampicin and fusidic acid. ß-lactam antibiotics are highly effective on peptidoglycan layer of the cell wall, and therefore Gram-positive organisms are much more sensitive to hydrophobic ß-lactams than the Gram-negatives. This effect is said to be due to the activity of porin proteins. Although these proteins act as non-specific diffusion channels, most hydrophobic molecules are not able to penetrate through these channels. In addition, by closing the channels, porins can reduce the permeability of the membrane to these molecules (Schindler and Rosenbusch 1981). The hight level resistance of *Pseudomonas aeruginosa* to a variety of antibiotics is suggested to be because of the closed porin channels (Nikaido and Vaara 1985). For ß-lactams even when they penetrate through the porins the periplasmic enzyme ß-lactamase is often available to inactivate the antibiotic.

C. Resistance to polycantionic agents:

Polycationic agents such as polymixins have great affinity towards cytoplasmic membrane. They attach to phospholipid content of this membrane and destroy it (Storm et al 1977). In most enteric bacteria the permeability barriers of the outer membrane particularly the LPS and protein structures were found to be the main factors of resistance to these agents (Vaara et al 1981). The molecule of polymixin B is too large to pass through the porins therefore is less effective than its related agents. It has been observed that the removal of the outer membrane increased the sensitivity of the organism to ploymixins up to 400 times (Teuber 1969).

The presence of different antibacterial inactivating enzymes in the periplasmic region is also an important protective factor for the bacterial cell.

The bacterial surface hydrophobicity is also another function of the outer membrane, protecting the organism against phagocytosis and body immune system and enhancing its invasive property, which will be discussed later.

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