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The cell envelope of tubercle bacilli

Mamadou Daffé*

Department of Tuberculosis and Infection Biology, Institut de Pharmacologie et Biologie Structurale (IPBS) du Centre National de la Recherche Scientifique (CNRS) and Université Paul Sabatier (Toulouse III), BP 64182, 205 Route de Narbonne, 31077 Toulouse, France

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SUMMARY

The envelope of tubercle bacilli and of other mycobacteria is important for the bacterial physiology since inhibition of the production of some of its constituents kills the cells. It consists of a plasma membrane, which is apparently homologous to plasma membranes of other bacteria, surrounded by a complex wall of carbohydrate and lipid, which is in turn surrounded by an outermost layer, called 'capsule' in the case of pathogenic species. The wall possesses a fundamental, covalently linked 'cell-wall skeleton' composed of peptidoglycan covalently linked to arabinogalactan esterified by very long-chain (up to C90) fatty acids (mycolic acids). These fatty acids form the inner leaflet of a typical outer membrane (mycomembrane) whose outer leaflet consists of a great variety of non-covalently linked lipids and glycolipids. The thickness of the mycomembrane is similar to that of the plasma membrane, implying dedicated conformations of mycolic acids. Finally, a periplasmic space also exists in mycobacteria, between the membrane and the peptidoglycan.

Mycobacteria are Gram-positive bacteria but the chemical nature of their envelope is different from those of both groups of Gram-positive and from Gram-negative bacteria. The lipid content of the cell envelope of mycobacteria may represent up to 40% of the cell dry mass, compared to less than 5% in other Gram-positive bacteria and only 10% in Gram-negative bacteria [9]. Such a lipid-rich coat could explain both the tendency of mycobacteria to grow in clumps and their distinctive property of acid-fastness. The permeability of cell walls of mycobacteria was found to be 10–100-fold lower than that of the notably impermeable bacillus *P. aeruginosa* [13]. This property justifies the occurrence of pore-forming proteins (porins) in the cell envelope of mycobacteria and related micro-organisms belonging to the order of *Corynebacteriales*. This unusual structure certainly makes it difficult for the host to damage the mycobacterial envelope, and while it is intact, the impermeable cell envelope protects the mycobacterium from damage.

The mycobacterial envelope consists of (i) a plasma membrane surrounded by (ii) a complex wall of peptidioglycan (PG) and arabinogalactan (AG) to which lipids are attached, which is in turn surrounded by (iii) an outer layer, called 'capsule' in the case of pathogenic species (Figure. 1A). Between the plasma membrane and the peptidoglycan is a periplasmic space.

1. The plasma membrane

The basic structure of the plasma membrane of the mycobacterial cell envelope does not seems to differ from that of the other plasma biological membranes, as judged from their appearance in ultra-thin sections, the characterization of classical metabolic functions and their known chemical composition. Isolated plasma membranes are typically obtained by breaking the cells by mechanical stress, *e.g.* sonication or shearing in the French pressure cell, followed by fractionation using differential centrifugation or density gradients (see [24]. No obvious difference was found between those of rapid- and slow-growing species examined (see [19]. Polar lipids, mainly phospholipids, assemble themselves, in association with proteins, into a lipid bilayer.

2. Cell wall

The wall of mycobacteria consists of a covalently linked 'cell wall skeleton' (CWS), and an abundant variety of wall-associated lipids (Figure. 2) and a few polypeptides, if any. The CWS is a giant macromolecule chemically composed of three covalently linked constituents: peptidoglycan, arabinogalactan (AG) and mycolic acids (Figure. 2), and defines the shape of the mycobacterial cell. It

* Corrersponding author.
E-mail address: mamadou.daffe@ipbs.fr.

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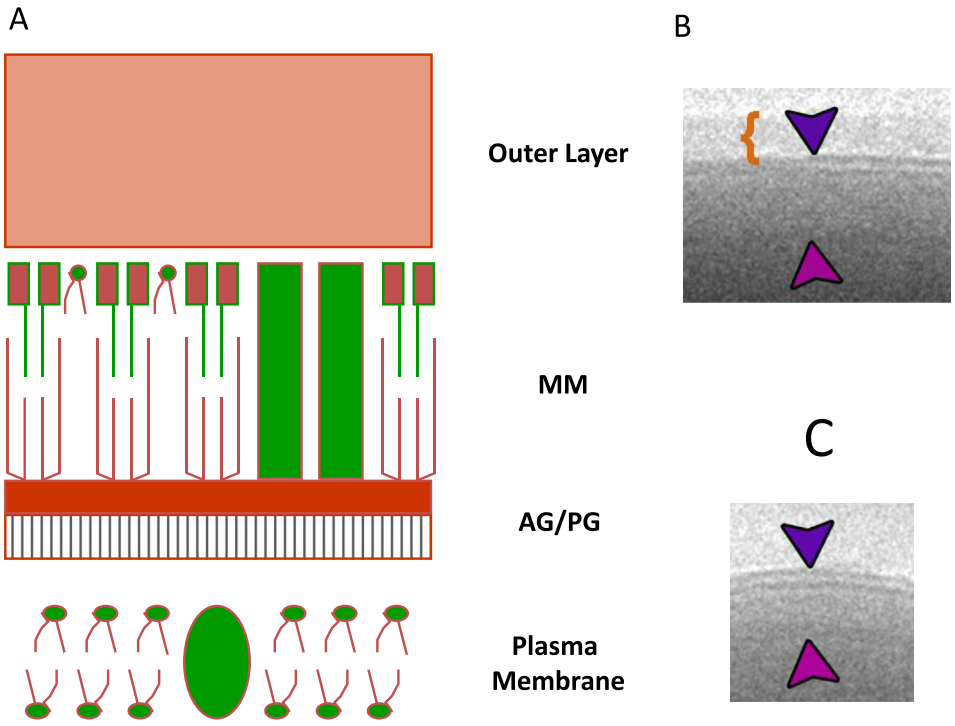

Figure 1. A) scheme of the mycobacterial envelope consisting of a plasma membrane (PM), surrounded by a complex wall of peptidioglycan (PG) and arabinogalactan (AG) to which lipids are attached (called mycomembrane, MM), which is in turn surrounded by an outer layer. Proteins are not represented in this cartoon, with notable exception of a representative integral protein of the PM and the cell wall pore-forming proteins. This model of the arrangement of the mycobacterial cell envelope is based on that of [19]; modified by Daffé and Draper [2]. B) and C) Cryo-electron micrographs of the cell envelope of mycobacteria grown in media without (B) and with (C) detergent (Tween80).

can be dissected into its constituent parts by relatively gentle methods, so that each part may be studied separately (see [4,17,18]. The peptidoglycan is composed of repeating units of N-acetylglucosamine and N-acetyl/glycollylmuramic acid cross-linked by short peptides. The arabinogalactan is a complex branched heteropolysaccharide that contains a galactan chain composed of alternating 5- and 6-linked D-galactofuranosyl residues; three D-arabinan chains substituted the D-galactan chain in *M. tuberculosis* (Figure. 2). In the tubercle bacillus, two thirds of the non-reducing termini pentaarabinosyl motifs are esterified by mycolic acids whereas half of these are occupied by mycoloyl residues in other mycobacterial species, including *M. leprae* and *M. bovis* BCG [18]. Mycolic acids (Figure. 2) are very long-chain (up to C90) α-branched and β-hydroxylated fatty acids esterified the four hydroxyl groups at position 5 of both terminal and 2-linked D-arabinofuranosyl of the pentaarabinosyl motifs.

The existence of an outer membrane, similar to that of Gram-negative bacteria, has been proved by Cryo-Electron Microscopy of VItreous Sections (Figure. 1B and C) [11,26,30]. This membrane, also called mycomembrane (MM), presumably contains the mycolic acid residues covalently attached to arabinogalactan and form its inner leaflet. The outer leaflet of the MM is likely constituted of various lipids, glycolipids and proteins, but the molecular composition of the MM still remains largely elusive. The thickness of this membrane, 7–8 nm, implies that the very long-chain mycolic acids adopt singular conformations, as those described by Villeneuve et.al., [29], to fit in the MM. The occurrence of a true permeability membrane, i.e. the MM, poses the problem of getting small polar molecules from the exterior, notably needed for nutrition. Gram-negative bacteria solve this problem by producing specialized proteins called porins, which form hydrophilic pores through the structure. Pore-forming proteins have been found in the walls of both slow- and rapid-growing mycobacterial species, including *M. tuberculosis* [28].

3. The outer layer

The outermost layer, which appears in infected cells as an electron-transparent layer (ETZ) represents a 'capsule' of polysaccharide and protein, plus in a few cases specialized glycolipids; it differs from the capsule around mycobacteria grown *in vitro* in being more extensive, presumably because the material that would be shed into the culture medium is retained by the phagosomal membrane [2]. Interestingly, mycobacteria embedded by cryo-microscopy show an extended capsule (Figure. 1B) [26] but the visualization of this layer greatly depends on the composition of the growth medium (Figure. 1B and C), e.g. the presence or absence of detergent [21,22,26], the treatments of the bacterial cells [22].

We have shown that *in-vitro*-grown tubercle bacilli are surrounded by an attached layer of polysaccharide [20] chemically similar to that present in the medium [15]. Growth of the cells as a pellicle apparently minimizes the loss of the capsule into the medium, presumably because it eliminates the mechanical stresses on the capsule caused by agitation of the medium [2]. Again, more extracellular material was recovered from the culture filtrates of the pathogenic species, e.g. *M. tuberculosis* and *M. kansasii*, than those of saprophytic and non-pathogenic strains such as *M. smegmatis* and *M. aurum* [15,16]. Shaking gently the bacterial pellicles with glass beads [20,22,23] extracts the outermost capsular compounds while the use of Tween 80 (Figure. 1B and C) leads to the progressive extraction of more material from expectedly deeper compartments [20,22,23,26]. The main components of the outermost capsular layer of *M. tuberculosis* are polysaccharides