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Lipid II: A central component in bacterial cell wall synthesis and a target for antibiotics

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A B S T R A C T

The bacterial cell wall is mainly composed of peptidoglycan, which is a three-dimensional network of long aminosugar strands located on the exterior of the cytoplasmic membrane. These strands consist of alternating MurNAc and GlcNAc units and are interlinked to each other via peptide moieties that are attached to the MurNAc residues. Peptidoglycan subunits are assembled on the cytoplasmic side of the bacterial membrane on a polyisoprenoid anchor and one of the key components in the synthesis of peptidoglycan is Lipid II. Being essential for bacterial cell survival, it forms an attractive target for antibacterial compounds such as vancomycin and several lantibiotics. Lipid II consists of one GlcNAc-MurNAc-pentapeptide subunit linked to a polyiosoprenoid anchor 11 subunits long via a pyrophosphate linker. This review focuses on this special molecule and addresses three questions. First, why are special lipid carriers as polyprenols used in the assembly of peptidoglycan? Secondly, how is Lipid II translocated across the bacterial cytoplasmic membrane? And finally, how is Lipid II used as a receptor for lantibiotics to kill bacteria?

1. Introduction

The bacterial cell wall is a unique structure. It consists mainly of a peptidoglycan polymer network located at the outside of the cytosolic membrane and it provides strength and shape to bacteria. A schematic presentation of the peptidoglycan layer and the biosynthesis of its building blocks is presented in Fig. 1. The units consist of the disaccharide GlcNAc and MurNAc to which a pentapeptide is attached. This unit is assembled on the cytosolic side of the membrane on a bactoprenol hydrocarbon chain via a pyrophosphate linker. The enzymes MraY and MurG catalyze the final assembly steps, resulting in the formation of Lipid II. This molecule translocates to the periplasmic side of the membrane, where the penicillin-binding proteins (PBPs) catalyze the insertion of peptidoglycan uniting into a growing cell wall. The lipid anchor carrying the pyrophosphate is shuttled back to the cytosolic side of the membrane, where it can be reused (after dephosphorylation to the mono-phosphate form) for the next round of synthesis.

All enzymes involved in peptidoglycan synthesis are known and various mechanistic aspects of the pathway are understood. This is not surprising since this pathway forms a prime target for antibiotics, of which penicillin and vancomycin probably are the best-known examples (Fig. 1). Both these antibiotics act on the periplasmic steps of the pathway involving Lipid II. This is also true for a subset of lantibiotics, which also target Lipid II and which will be discussed later. Despite considerable knowledge of cell wall synthesis several key questions remained unanswered so far. It is the aim of this paper to identify some of these key questions and to discuss and speculate on possible answers.

The specific questions to be addressed are:

1. Why did nature choose polyprenols as lipid anchors for the peptidoglycan units?
2. How are Lipid II molecules translocated from the cytosolic to the periplasmic leaflet of the membrane?
3. How can lantibiotics in such a versatile manner use Lipid II as receptor to kill bacteria?

Before these questions are addressed, it is useful to provide some more in-depth insight into Lipid II.

2. Lipid II occurrence and properties

Lipid II is a minor component of the bacterial cytoplasmic membrane. Estimates for Gram-positive bacteria typically give values below 1 mol% of the membrane phospholipids [1]. This small amount of Lipid II is responsible for the fast growing bacterial cell wall. It can be estimated that the entire Lipid II cycle will take somewhat less than 1 s, which immediately implies that most steps of the cycle will have even higher turnover numbers.

The chemical structure of Lipid II and a space-filling model are presented in Fig. 2. It shows a large hydrophilic head group linked via a pyrophosphate to a very long bactoprenol chain. Given the

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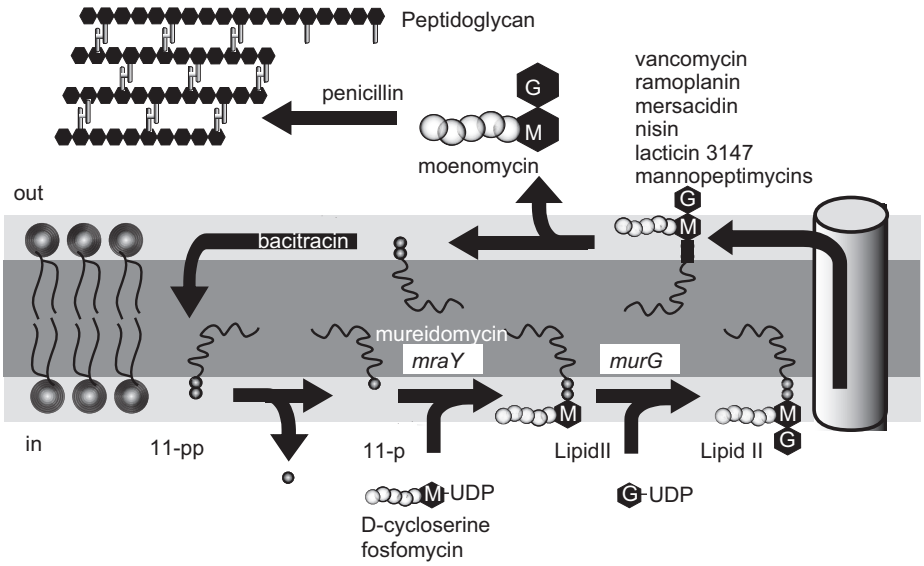

Fig. 1. Membrane events in the bacterial cell wall synthesis cycle. See text for details. Additionally, antibiotics are placed at the position corresponding to the step in the cycle that they inhibit. The antibiotics penicillin and moenomycin block the transpeptidase and transglycosylase activity of PBPs, respectively. The antibiotics vancomycin to mannopeptimycins specifically target Lipid II, bacitracin binds undecaprenyl phosphate and thus inhibits recycling of the polyisoprenoid anchor, D-cycloserine and fosfomycin inhibit the synthesis of UDP-MurNAc-pentapeptide and mureidomycin specifically inhibits MraY.

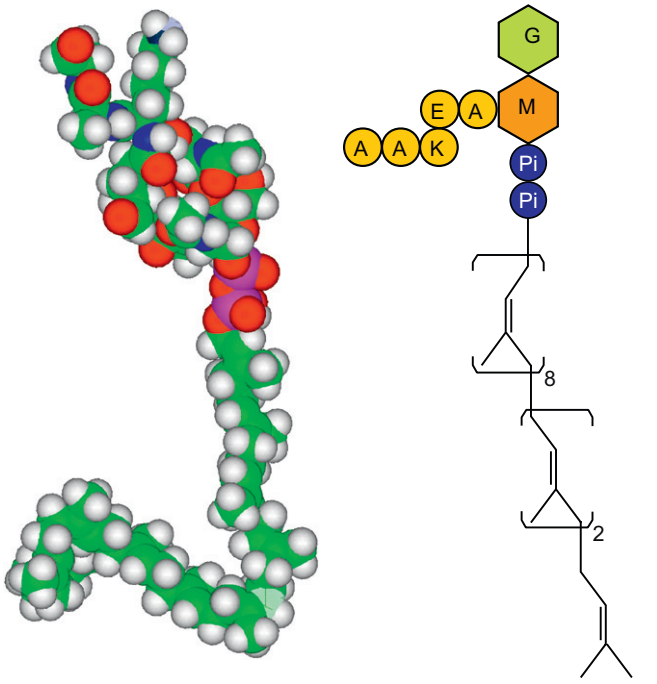

Fig. 2. Chemical structure and space-filling model of Lipid II. The specific configurations of the isoprene units are shown in the right structure, as well as the typical linkage (via the side chain) between the glutamate and lysine of the pentapeptide.

complexity of the molecule it is not surprising that the total chemical synthesis was only recently accomplished. Fortunately, a semi-synthetic production method of Lipid II has been described [2] that allows flexible production of Lipid II and its analogues in quantities sufficient for biochemical and biophysical research.

Incorporation of Lipid II in supported bilayers of phosphatidylcholine revealed that the bactoprenol chain must be fluid, because Lipid II partitions in fluid domains, present in mixed solid/fluid bilayers. This was observed by atomic force microscopy (AFM) using the lantibiotic nisin, which binds specifically to Lipid II to visualize its location [3]. Consistent with this finding was the observation made by confocal fluorescence microscopy that NBD-labeled Lipid II was homogeneously distributed in GUVs of DOPC [4].

The AFM technique also revealed that the head group of Lipid II is soft and can be penetrated by the AFM probe [3]. This property of the molecule allowed insight into the dimensions of the head group, which was estimated to be 1.9 nm high as compared to the level of the phospholipid head group [3]. Given that the limiting area of the head group is 1.5 nm^2 as observed in monolayer studies, the pentapeptide must have an upright position and be fully accessible to the aqueous phase [3].

Another intriguing property of Lipid II and several of its precursors is that it displays a pyrophosphate in the membrane interface. This could readily serve as a binding motive for the proteins involved in the Lipid II cycle. In the forthcoming sections it will be described that the pyrophosphate of Lipid II forms the binding motive for a class of lantibiotics.

3. Why polyprenols as lipid anchors for the peptidoglycan units?

Typical bacterial membrane lipids have alkyl chains as hydrophobic moieties. However, the bactoprenols, being polyisoprenoids, are rare but functionally very important constituents of the hydrophobic interior of the bacterial membrane. This immediately raises the question as to why bactoprenol acts as lipid anchor for the peptidoglycan unit instead of the more conventional alkyl chains. Before discussing this question it is useful to consider the biological processes that make use of polyprenol-linked precursors. Within the bacterial membrane the bactoprenols, next to their role in peptidoglycan synthesis, also fulfill carrier functions in the synthesis pathways of other complex (poly)saccharides such as the O-antigen of LPS, oligosaccharides involved with N-linked protein glycosylation in *Campylobacter jejuni* or the teichoic acids of Gram-positive bacteria [5–7].

Interestingly, a striking parallel can be noticed between bacterial peptidoglycan synthesis and *N*-glycosylation in eukaryotic cells. *N*-glycosylation takes place on the luminal site of the endoplasmic reticulum and involves the transfer of a large oligosaccharide unit to specific asparigine residues during protein translocation [8–11]. The mannose-rich oligosaccharide is linked via a pyrophosphate to a long polyprenol chain of the dolichol