Poster Presentation

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Biophysical characterization of enzymes from the lipid A biosynthesis pathway

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The outer membrane of Gram-negative bacteria possesses an inner leaflet composed primarily of phospholipids while the outer leaflet contains both phospholipids and lipopolysaccharide (LPS). LPS acts as a structural barrier that protects Gram-negative bacteria from antibiotics and other environmental stress. It contains a Lipid A moiety which is a glucosamine-based saccharolipid. Lipid A is the active component of the bacterial endotoxin and strongly modulates the human immune response.

Lipid A structure determination coincided with the discovery and structural characterization of the precursor 2,3-diacylglucosamine 1-phosphate or "lipid X" [1]. Lipid X is biosynthesised in the cytoplasm of the bacterium by the enzyme LpxH which cleaves UMP from UDP-2,3-diacylglucosamine. The enzyme LpxB catalyses the formation of a β,1′-6 linked disaccharide by condensing the monosaccharide product and substrate of the LpxH reaction (Fig 1).

LpxB and LpxH are characterized as peripheral membrane proteins. LpxB is an inverting glycosyl transferase of the GT-B superfamily and a member of CAZy database family 19. LpxB is a target for the development of new antibiotics, but no member of LpxB orthologues have been characterized mechanistically or structurally. LpxH catalyzes the first membrane-associated step of the Lipid A biosynthetic pathway. LpxH occurs in 70% of clinically important Gram-negative pathogens. The structures of LpxH from P. aeruginosa and H. influenzae in complex with the product lipid X, were recently determined [2,3]. Both the structures show a unique insertion lid that covers the di-manganese cluster at the active site.

The presence of the bound product in the LpxH structures may suggest that LpxB is required to release lipid X from LpxH concurrently with the binding of UDP-2,3-diacylglucosamine for the formation of the disaccharide. Here we present the expression, purification and biophysical characterization of both LpxB and LpxH. We also aim to find suitable conditions to crystallise these proteins as a complex and solve the structure by X-ray crystallography.

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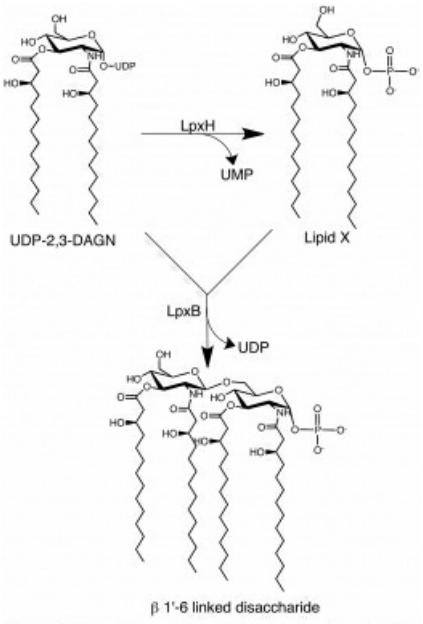

Figure 1. Enzymatic reactions catalysed by LpxH and LpxB

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