X-ray Crystallography of LpxB, a Glycosyltransferase in the Lipid A Synthesis Pathway

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Lipid A is the membrane anchor of lipopolysaccharide (LPS), the major component of the outer leaflet of the Gram negative outer membrane. LPS helps protect Gram negative bacteria from hydrophobic toxins, such as many antibiotics, thereby contributing to the growing problem of antibiotic resistance. In addition, lipid A (endotoxin) can overstimulate the innate immune system during sepsis resulting in septic shock. Sepsis causes 200 thousand deaths annually in part due to the toxic effects of lipid A. Therefore, the lipid A synthesis (Raetz) pathway is an attractive target for the development of new broad spectrum antibiotics that could be used to treat sepsis and other resistant bacterial infections. LpxB is the last highly conserved enzyme in the Raetz pathway and is one of two enzymes of the canonical Raetz pathway that have not been structurally characterized. Thus, the aim of this project has been to obtain crystal structures of LpxB. High resolution structures of solubilized LpxB were obtained bound to the substrate analogue UDP-N-acetyl-glucosamine and in the apo state. LpxB forms two Rossmann-like domains as is typical for the Glycosyltransferase B family; however, LpxB forms a unique dimer with the C-termini of the polypeptides swapped between the subunits. The location of the nucleotide-binding pocket confirms that LpxB binds the donor/electrophile substrate on the C-terminal side of the active site cleft. The predicted catalytic base (D98), which activates the acceptor/nucleophile substrate, is located on the N-terminal side of the cleft. Hydrophobic residues in a predicted membrane-binding patch were shown to be important, but not essential, for activity. This structure may aid in the design of new antibiotics against Gram negative bacteria.