BIOCHEMICAL AND STRUCTURAL ANALYSIS OF THE ROLE OF THE *wlb* GENE LOCUS IN *BORDETELLA PERTUSSIS* LIPOPOLYSACCHARIDE BIOSYNTHESIS

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INTRODUCTION. *Bordetella pertussis* is the causative agent of whooping cough in children and chronic cough in adults. *Bordetella* lipopolysaccharide (LPS) has been implicated in the infection process and has been shown to be highly immunogenic, and acts as an immunological adjuvant which displays the properties of an endotoxin. Tentative gene function assignments have been made for the *B. pertussis* LPS band A trisaccharide biosynthetic locus (*wlb*), albeit based on rather weak sequence similarities[1,2]. The *B. pertussis wlbD* gene product is a putative uridine-5-diphosphate N-acetylglucosamine 2'-epimerase based on its homology to *E. coli rffE* (32% identical), an established UDP-GlcNAc 2'-epimerase that is involved in Enterobacterial Common Antigen (ECA) formation. The key active site residues in *rffE* are present in the *wlbD* sequence.

RESULTS AND DISCUSSION. The *wlbD* gene from *B. pertussis* has been cloned and over-expressed in *E. coli* and the resulting protein has been purified to homogeneity. All the activity assays failed to show the epimerase activity *in vitro* and *wlbD* failed to complement the *E. coli rffE* mutant. This suggests that assignment of *wlbD* is almost certainly wrong, which prompts re-examination of the entire biosynthetic pathway (see Fig.1). The most likely substrate for *wlbD* may be UDP-Glc-2,3-diNAc. The 3-D structure of *wlbD* may throw light on its function, crystals of the mutant Gln339Arg *wlbD* enzyme have been obtained in the presence and absence of UDP-GlcNAc and 1.6 Å (in Grenoble, France) and 2.3 Å (in house) data sets were collected, respectively.[4] In order to solve the structure a Seleno-methionine variant protein was prepared and MAD data were collected since molecular replacement using *rffE* as model failed. The crystal structure exists as a dimer and each monomer has two domains (see Fig. 2). The *wlbD* structure shares marked topological similarity to the *rffE* structure and other glycosyltransferases (DALI analysis). Further structural analysis is going on.

FIGURE 1. (a) Original proposed pathway; (b) revised pathway.

FIGURE 2. Crystal structure of *wlbD*.

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