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Expression, essentiality, and a microtiter plate assay for mycobacterial GlmU, the bifunctional glucosamine-1-phosphate acetyltransferase and *N*-acetylglucosamine-1-phosphate uridyltransferase

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ABSTRACT

UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc) is an essential precursor of peptidoglycan and the rhamnose-GlcNAc linker region of mycobacterial cell wall. In *Mycobacterium tuberculosis* H37Rv genome, Rv1018c shows strong homology to the GlmU protein involved in the formation of UDP-GlcNAc from other bacteria. GlmU is a bifunctional enzyme that catalyzes two sequential steps in UDP-GlcNAc biosynthesis. Glucosamine-1-phosphate acetyl transferase catalyzes the formation of *N*-acetylglucosamine-1-phosphate, and *N*-acetylglucosamine-1-phosphate uridylyltransferase catalyzes the formation of UDP-GlcNAc. Since inhibition of peptidoglycan synthesis often results in cell lysis, *M. tuberculosis* GlmU is a potential anti-tuberculosis (TB) drug target. In this study we cloned *M. tuberculosis* Rv1018c (*glmU* gene) and expressed soluble GlmU protein in *E. coli* BL21(DE3). Enzymatic assays showed that *M. tuberculosis* GlmU protein exhibits both glucosamine-1-phosphate acetyltransferase and *N*-acetylglucosamine-1-phosphate uridylyltransferase activities. We also investigated the effect on *Mycobacterium smegmatis* when the activity of GlmU is fully removed or reduced via a genetic approach. The results showed that activity of GlmU is required for growth of *M. smegmatis* as the bacteria did not grow in the absence of active GlmU enzyme. As the amount of functional GlmU enzyme was gradually reduced in a temperature shift experiment, the *M. smegmatis* cells became non-viable and their morphology changed from a normal rod shape to stubby-rounded morphology and in some cases they lysed. Finally a microtiter plate based assay for GlmU activity with an OD_{340} read out was developed. These studies therefore support the further development of *M. tuberculosis* GlmU enzyme as a target for new anti-tuberculosis drugs.

1. Introduction

The emergence of drug resistant strains of bacteria has become a very serious problem in controlling infectious disease (Peterson, 2005). This is especially true in the case of tuberculosis. Tuberculosis (TB) remains a major threat to world health; the world-wide statistics of eight million people developing active tuberculosis and nearly one

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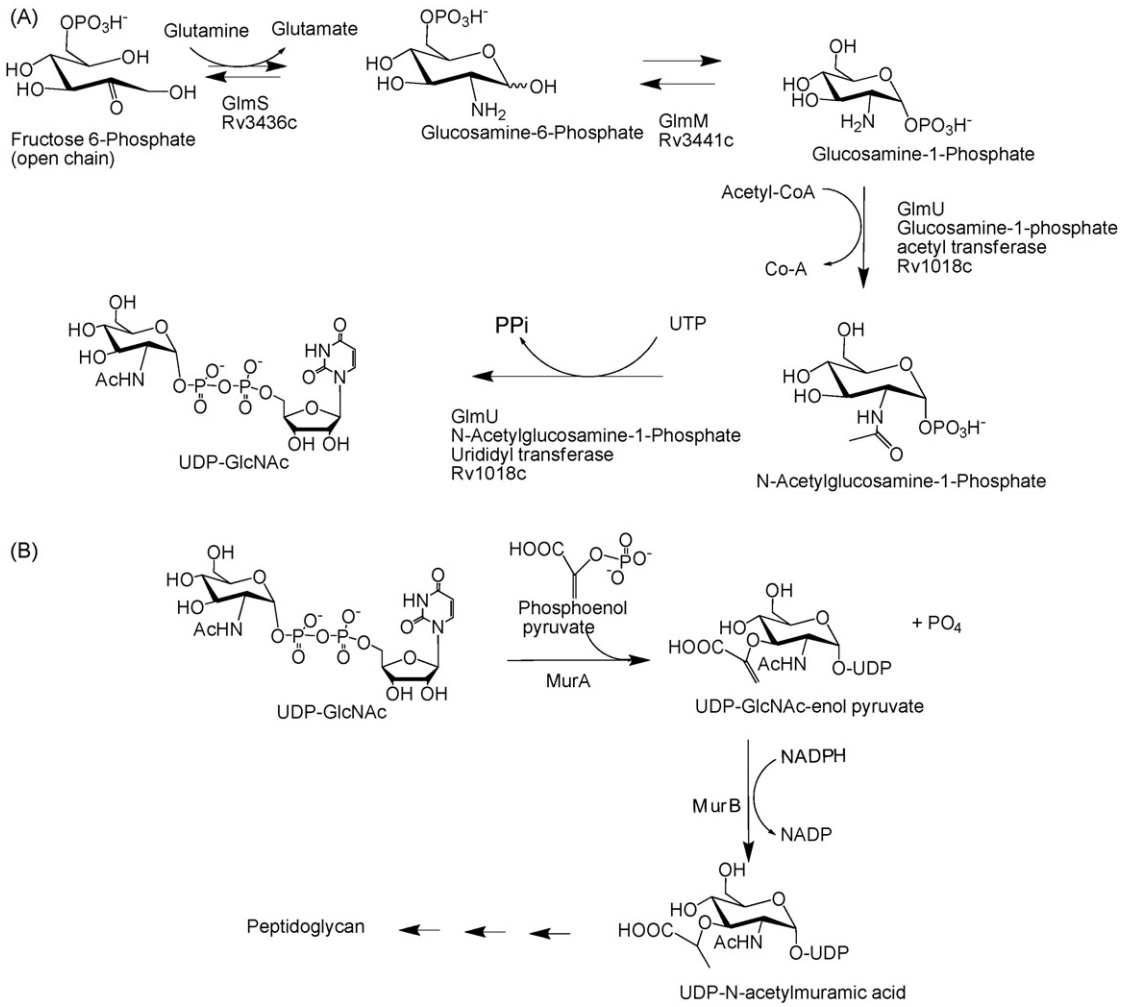

Fig. 1. (A) Biosynthetic pathway of UDP-GlcNAc formation in mycobacteria. GlmU (Rv1018c) is a bifunctional enzyme which has glucosamine-1-phosphate acetyl transferase and *N*-acetylglucosamine-1-phosphate uridylyl transferase activities. (B) The reactions of MurA and MurB which act on the UDP-GlcNAc product of GlmU and are used in the linked assay in which the loss of NADPH is monitored. In the bacterium, the product, UDP-MurNAc, is then converted to peptidoglycan.

fourth of these dying from this disease is a stark reality (Dye et al., 1999). (See also the Global Alliance for TB drug Development at www.tballiance.org) TB, as a public health problem, has been complicated by the lack of a wide array of chemotherapeutics against its causative agent, *Mycobacterium tuberculosis*, and hence, the emergence of drug resistant strains to the few front-line drugs has been a large problem in recent years. Although current first-line anti-TB drug regimens can achieve more than 99% efficacy, this is often reduced because of drug resistance (O'Brien and Nunn, 2001). Multiple drug resistant TB (MDR-TB), where the bacillus is resistant to several first-line drugs used to be the most fearsome type of resistance; now, however, strains resistant to both first and second line antibiotics are appearing (Gandhi et al., 2006; Van Rie and Enarson, 2006); these have been dubbed extreme drug resistant TB (XDR-TB).

The mycobacterial cell wall core consists of two layers (McNeil and Brennan, 1991; Lee et al., 2006). The highly impermeable outer layer is composed of mycolic acids which are lipids containing 70–90 carbons. The inner layer consists of peptidoglycan. These two layers are covalently tethered via the connecting polysaccharide arabinogalactan (McNeil and Brennan, 1991; Lee et al., 2006), which is attached to the peptidoglycan through a disaccharide linker, α-L-rhamnosyl-(1,3)-α-D-GlcNAc-(phosphate). Thus, GlcNAc is an essential component of both peptidoglycan (as in most bacteria) and the rhamnose-GlcNAc linker of the cell wall in mycobacteria.

In *E. coli* (Mengin-Lecreulx and Vanheijenoort, 1994; Riley et al., 2006), three enzymes catalyze four sequential steps in the biosynthesis of UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc), an activated nucleotide sugar of GlcNAc. The glutamine fructose-6-phosphate transferase, encoded by the *glmS* gene, catalyzes fructose-6-phosphate and glutamine to glucosamine-6-phosphate and glutamate, the phosphoglucosamine mutase, encoded by the *glmM* gene, converts glucosamine-6-phosphate ($GlcNH_2$-6-P)