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KdsB Kinetics Measurements and Effect of kdsB Mutation on biosynthesis of Lipopolysaccharide and K1 capsule of Escherichia coli

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Abstract

The minimum prerequisite for a viable lipopolysaccharide (LPS) structure in all Gram-negative bacteria is a lipid A molecule which has been inserted into the outer membrane and substituted with two residues of Kdo. The substitution of lipid A with Kdo entails, initially, the activation of Kdo by its addition to CTP, forming CMP-Kdo. The formation of CMP-Kdo is catalysed by the transferase enzyme CMP-Kdo synthetase, KdsB. Mutations in kdsB are lethal with a lack of Lipid A in the outer membrane, so it represents a potential target for new antimicrobials. Ongoing resistance of Gram-negative bacteria to current antibiotics require the generation of new antimicrobials against novel targets. The first step to design a potentially new Gram-negative antimicrobial was the purification and kinetics evaluation of KdsB. KdsB purification was performed initially using HisTrap HP His-tag protein purification columns followed by AKTA purifier performing size exclusion chromatography. The KdsB molecular weight and concentration was determined using multi angels light scattering (MALS) to be 60.4 kDa as a dimer and 2.5 mg/ml. The kinetic properties of the purified KdsB were quantified spectrophotometrically using the linked pyrophosphate assay. Vmax of the reaction was 2.0 0.1 µMmin-1, the Km of Kdo was calculated to be 100 0.3 µM, and the Km of CTP was quantified to be 5 0.1 µM. The kpsU gene encodes CMP-Kdo synthetase within group 2 capsule gene clusters such that E. coli strains expressing group 2 capsules have two functional CMP-Kdo synthetase enzymes. Therefore, it should be possible to generate a non-lethal kdsB mutant in such strains at a capsule permissive temperature such as 37◦C to use it as a screen to identify repressors of capsule transcription at capsule nonpermissive temperature like 20◦C. The first aim to construct a kdsB mutant in strain EV1 was achieved. As predicted the growth at 37◦C for both the wild type and the kdsB mutant was identical with no detectable phenotype including K1 capsule expression.

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