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Combatting antibiotic resistance in Neisseria gonorrhoeae by targeting the final enzyme in cysteine biosynthesis, CysK

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Background

Cysteine plays a vital role in protein folding and function, and synthesis of glutathione for protection against oxidative stress during infection, making it a promising new target for development of antimicrobials. O-acetylserine sulfhydrylase (OASS) catalyses the final step of the two-step cysteine biosynthesis pathway. The first enzyme in the cysteine biosynthesis pathway (CysE) combines with CysK to form the cysteine synthase complex (CSC) in many bacteria and is hypothesised to play an important role in sulfur flux. Most bacteria have two isoforms of OASS that produce cysteine, sulfide and O-acetylserine utilising OASS A/CysK, and thiosulphate utilising OASS-B/CysM. N. gonorrhoeae has only the OASS-A/CysK isoform in its genome.

Aim/Methods

This research aims to biochemically characterise the CysK enzyme kinetically and structurally. Kinetics were determined via a stopped assay method which colourimetrically measures cysteine produced. Structural characterisation was achieved using X-ray crystallography through the Australian Synchotron.

Results

Kinetic characterisation demonstrates CysK has OASS activity, displaying positive cooperativity with respect to substrates, O acetylserine and sulphide. Sulphide shows partial allosteric inhibition, and thiosulphate is not used as a substrate. The CysK structure was solved to 2.49 Å and shows CysK belongs to the tryptophan synthase β superfamily and adopts a homo-dimeric structure consisting of two monomers. Positive cooperativity is supported as co-factor binding residues are in inactive and active conformations in each monomer of the dimer, respectively. Preliminary data indicates that the CSC does not form in N. gonorrhoeae.

Conclusions

The kinetic and structural profile of CysK have been determined, confirming the identity of the OASS present in N. gonorrhoeae as a CysK isoform that is unable to utilise thiosulphate as a sulphur donor and shows structural similarity to other CysK enzymes. Kinetic and structural data has enabled virtual screening to be completed for potential inhibitors. We have obtained 10 initial inhibitors to test using the colourimetric activity assay. Future research includes characterising our cysK gene knockout from N. gonorrhoeae, and its role in infection and adhesion to mammalian cells. Metabolomics will be undertaken to determine the in vivo role of CysK and further inform our understanding of sulfur metabolism in N. gonorrhoeae.