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Targeting serine acetyltransferase from Neisseria gonorrhoeae; structural and biochemical basis of inhibition

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Background

Over decades of antibiotic use, antimicrobial-resistant Neisseria gonorrhoeae strains have emerged at an alarming rate. To combat antimicrobial-resistant N. gonorrhoeae requires the development of new antimicrobials. Biosynthesis of the amino acid, L-cysteine, has been identified as a promising pathway for developing new antimicrobials. Cysteine not only plays a structural role in protein folding, but is also a precursor for redox compounds, such as glutathione, which are key for N. gonorrhoeae to mitigate oxidative stress. Serine acetyltransferase (CysE) catalyses the first step in the cysteine biosynthesis pathway and acts a key point of regulation for maintaining intracellular cysteine concentrations. CysE has been identified as an essential gene in N. gonorrhoeae and given the absence of this pathway in humans, makes this enzyme a promising target for antimicrobial development.

Aim/Methods

We have structurally and kinetically characterised CysE from N. gonorrhoeae using several biochemical techniques. Using X-ray crystallography, we have determined the structure of the CysE with and without substrate bound. CysE kinetic activity has been characterised through measuring the depletion of substrate L-serine. CysE inhibition was characterised through IC50 and mode of inhibition assays.

Results

Structural insights show that CysE has conserved structural features and active site residues, consistent with the hexapeptide acyltransferase family. Each structure displays both extended and closed conformations of the C-terminal tail, crucial for the formation of the cysteine synthase complex. Kinetic characterization demonstrates that CysE exhibits serine acetyltransferase activity and is subject to feedback inhibition by cysteine, where it competitively inhibits CysE relative to both substrates, L-serine and acetyl-CoA.

Conclusions

Overall, these data show that CysE from N. gonorrhoeae is a functional serine acetyltransferase and is regulated via feedback inhibition by the pathway product, cysteine. These findings have provided the structural and mechanistic basis for inhibitor development. Using computational inhibitor screening we have identified promising hit inhibitors that are currently being tested using in vitro enzymatic assays.