Abstract 1737

Diadenosine Polyphosphatases of the NUDIX Hydrolase Superfamily in *M. tuberculosis* and *M. leprae*

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M. tuberculosis contains 11 potential Nudix hydrolases, and we are characterizing these enzymes as potential novel antibiotic targets. The diadenosine polyphosphatases (ApnAases)/mRNA decapping enzymes are a family of enzymes within the Nudix hydrolase superfamily. In *M. tuberculosis* there is the primary Nudix ApnAase and the secondary Nudix ApnAase. There are also orthologs of these two ApnAases in *M. leprae*. The diadenosine polyphosphatases from *Legionella pneumophilia* and *Bartonella bacilliformis* have been found to be important in each pathogen's ability to invade its host cells. It is of interest to know whether these enzymes act in the same way in *M. tuberculosis* and *M. leprae*. If they are all found to be involved in invasiveness and thus in virulence, then these enzymes could be novel antibiotic targets. We have cloned and overexpressed each protein and have subcloned each into a HisTag vector to optimize purification. The *M. tuberculosis* enzymes have been purified and characterized, and the primary ApnAase is in the process of being crystalized for structure determination. The *M. leprae* enzymes express too insolubly to purify and characterize, and thus we are working on increasing the expression of soluble protein so that we can study these enzymes as well; currently we know that they each have ApnAase activity (in the crude extract) above that of *E. coli* enzymes alone.

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Abstract 1745

Examining the Ability of Gut Bacteria to Metabolize Metformin

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Metformin is the drug most frequently prescribed for type 2 diabetes worldwide. This medication has been shown to alter the gut microbiome in diabetic patients, improving glucose metabolism. Metformin has been touted as having antiviral and anti-aging properties, making it a possible treatment option for other medical conditions. Studies have shown that 30% of patients who take metformin get sick and of those 5% discontinue the use of the drug. The mechanisms by which metformin alters the gut microbiome and causes side effects are poorly understood. The goal of this research was to investigate whether gut bacteria could metabolize metformin. If bacteria in the gut microbiome can degrade metformin, the degradation byproducts may alter the gut microbiota and cause patients to become ill. We hypothesized that bacteria in the human gut have proteins homologous to metformin degradation enzymes and can thus degrade metformin when it is used as a nitrogen source for growth. Bioinformatics analyses were conducted to identify proteins similar metformin degrading enzymes in the human gut microbiome. Two gene sequences, one from Blautia hydrogenotrophica and one from Lachnospiraceae symbiosum, were chosen for further study because they shared 20–30% sequence similarity to GbuAB, an enzyme that degrades metformin to guanylurea. The genes were cloned and over-expressed in *E. coli* to test these proteins'capacity to degrade metformin. Enzyme activity was tested by incubating the cell lysates with metformin, arginine, agmatine and 4-guanidino-butyric acid. HPLC analysis revealed no metformin degradation in the Lachno and Blautia lysates. A urea released assay showed these lysates degraded agmatine to urea with a specific activity of 10 umol/min/mg of protein. Growth studies to evaluate degradation of metformin when used as the nitrogen source were carried out using two different brands of commercial probiotics to model gut bacteria. The HPLC results show limited metformin degradation by these cultures. Taken together, these results indicate that gbuAB homologs from gut bacteria encode functional agmatinases rather than metformin-degrading enzymes, and that commercial probiotics have limited ability to degrade metformin; the absence of known metabolic intermediates suggests that the drug is not fully metabolized. Evidence from this work suggests that side effects experienced by patients taking metformin are not caused by byproducts of the drug's microbial degradation.

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