Kinetic characterisation and mathematical modelling of coenzyme A biosynthesis salvage pathway in *M.tuberculosis*

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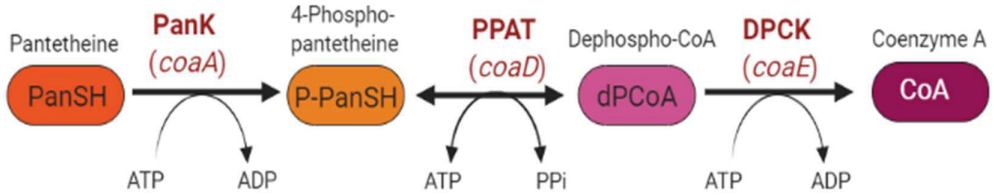

Figure 1: Schematic of the coenzyme A salvage pathway in Mycobacterium tuberculosis showing the intermediates and enzymes involved. This pathway converts pantetheine to Coenzyme A in a three step process. The proteins involved are Pantothenate kinase (PanK), Phosphopantetheine adenylyltransferase (PPAT) and Dephosphocoenzyme A kinase (DPCK).

Introduction: Tuberculosis, an infectious disease prevalent in developing countries, is a major concern due to the increasing failure of known antibiotics to treat this disease. This is due to *Mycobacterium tuberculosis*, the bacterium responsible for tuberculosis (TB), becoming increasingly resistant to the current arsenal of anti-TB drugs, leading to an increase in multi-drug resistant disease cases and death. There is an urgent need for the discovery of new anti-TB drugs that target pathways different to the current treatments. A possible target is the biosynthesis salvage pathway of Coenzyme A (CoA). By constructing, validating and analysis of a mechanistic mathematical model for this pathway based on the intrinsic characteristics, a rational approach is used for drug target identification in the pathway.

Methodology: The coenzyme salvage pathway in *M. tuberculosis* uses three enzymes to convert pantetheine to CoA. These three proteins were expressed, purified and kinetically characterised to construct detailed parameterised rate equations. With these rate equations a computer model was constructed using ordinary differential equations. The model was validated by testing its predictive capacity of intermediate dynamics in a reconstructed *in vitro* pathway.

Results: A detailed kinetic model for the three enzyme pathway was constructed and validated. The workflow for the construction uses classic initial rate kinetics as a first step, followed by progress curve analysis for the individual enzymes and a final modelling of intermediate dynamics for the complete reconstituted pathway. Reconstitutions were made with enzyme ratios chosen to reflect in vivo enzyme ratios and it was observed that not the PanK which is usually indicated as “the rate-limiting-step” but rather DPCK seems to have the highest control.

Discussion and Conclusion: The use of mechanistic models built on kinetic parameters determined in isolation can be used to predict the workings of metabolic pathways with varying inputs. The model can be used to simulate the expected outcome of changes in substrates, inhibitors and protein concentrations. Initial results indicate that the last enzyme in the pathway could potentially be the best drug target in the pathway.

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