Structure of *Mycobacterium tuberculosis* tryptophan synthase: a model system for allosteric inhibition

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The survival of many bacteria, including pathogenic species, depends on the ability to synthesize the amino acid L-tryptophan whenever it is not available from the environment. The pathway for L-Trp biosynthesis is typically organized in a single strictly regulated *trp* operon and most often include *trpE, trpG, trpD, trpC, trpB* and *trpA* genes. The *trpA* and *trpB* genes encode α and β subunits of a tetrameric $\alpha_2\beta_2$ tryptophan synthase (TrpAB), catalyzing the last two reactions of L-Trp biosynthesis. The tryptophan biosynthetic pathway was validated as conditionally essential for the survival of *Mycobacterium tuberculosis* (Mtb) during infection. This pathway is absent in humans and has a high potential for anti-tuberculosis drug targets. We have expressed, purified and crystallized active $\alpha_2\beta_2$ TrpAB from Mtb. A novel ligand was identified at the Broad Institute using library screening as a growth inhibitor of Mtb and targeting TrpA. We have determined several crystal structures of the enzyme in apo-form and in complex with a new inhibitor. The results of structural studies combined with functional characterization shows that the ligand is an allosteric inhibitor and binding in a highly unique fashion at the interface between TrpA and TrpB. Unexpectedly, though the compound potently inhibits the overall production of tryptophan, it increases the enzyme's affinity for substrates and stabilizes closed, active conformation of TrpB. In contrast to substrate mimetics, it affects multiple steps of the TrpAB reaction, drastically slowing the rate of both the TrpA and TrpB reactions. The new ligand represents a useful probe for understanding Mtb TrpAB regulation, function, and catalytic mechanism. Our findings suggest that allosteric inhibition can be a powerful strategy for targeting metabolic enzymes, and support the effectiveness of such strategies targeting functions that are required for *in vivo* infection, despite their apparent dispensability in the presence of tryptophan under *in vitro* conditions.

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