Shikimate Kinase: Potential Target for New Antibacterial Agents

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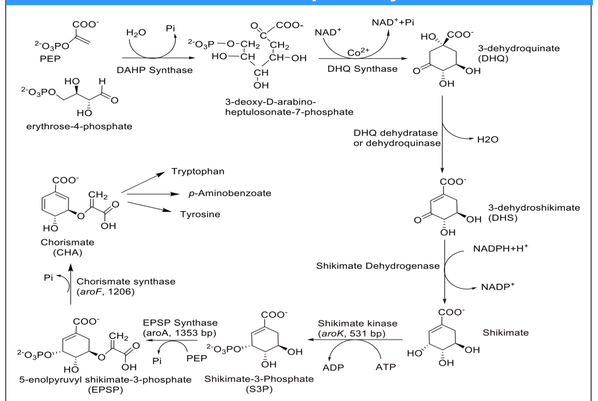
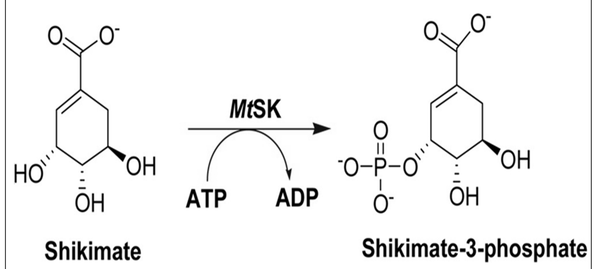
Abstract

Tuberculosis is one of the world's leading cause of mortality from a single bacterial pathogen, with over 10 million reported cases each year. There is an alarming increase in the prevalence of drug-resistant strains, thus the need for the discovery of novel anti-tubercular agents. *Mycobacterium tuberculosis* shikimate kinase (*Mt*SK) catalyzes the 5th step of the shikimate pathway, converting shikimate to shikimate-3-phosphate. The overall goal of this project is to express and characterize *Mt*SK and screen for potential anti-tubercular agents. Transformation of XL-1 blue competent cells was performed using a pET 21b plasmid with *aro*K gene inserted at the multiple cloning site. Plasmids were cloned and purified and used to transform BL 21 DE3 competent cells for subsequent protein expression. With further large-scale expression, purification and characterization, *Mt*SK kinetic parameters would be determined prior to enzyme inhibition studies using inhibitors like avarone, a marine sponge. sesquiterpene quinone and derivatives thereof.

Shikimate pathway

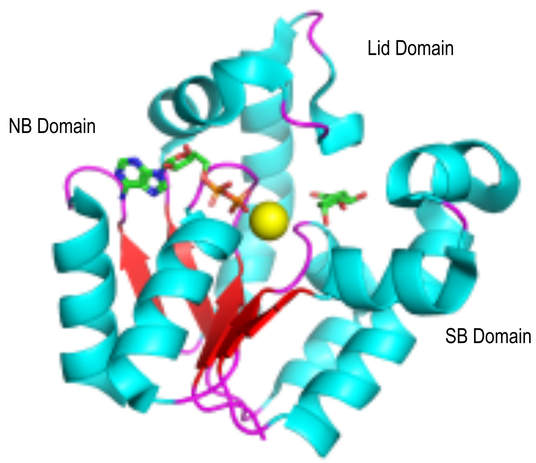


Shikimate Pathway: 5th Step



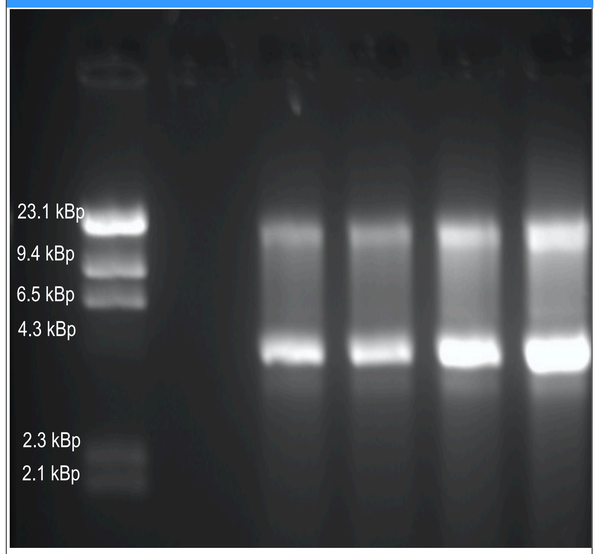
The 5th step of the shikimate pathway catalyzed by shikimate kinase. The enzyme phosphorylates shikimate, using its co-substrate ATP.

Shikimate Kinase (SK)



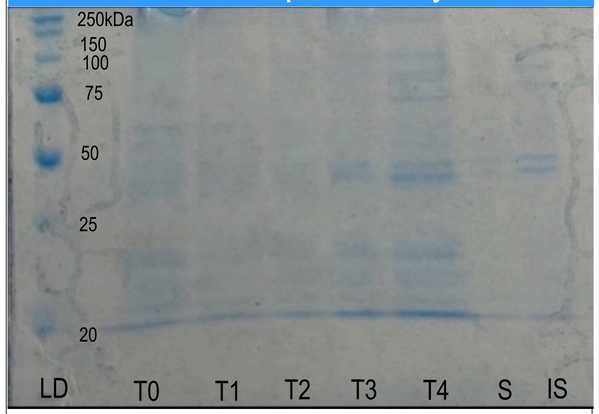
Mycobaterium tuberculosis shikimate kinase (*Mt*SK). PDB acc. 1WE2. The structure shows Nucleotide binding domain (NB), the conformationallly flexible Lid domain and the substrate binding domain (SB)

First Transformation: XL-1 Blue cells



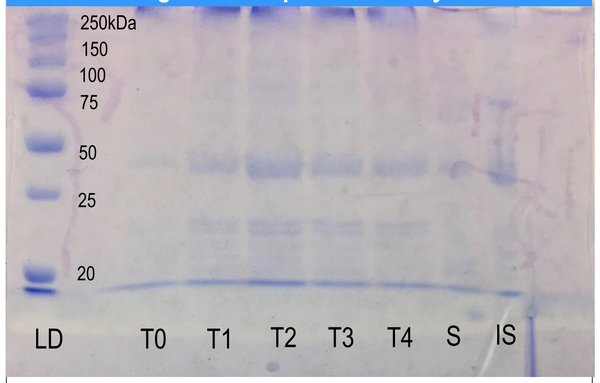
Purified plasmids migrated and banded at approximately 4.1 kBps, suggesting the presence of pET21b with incorporated *aroK* gene.

Small-scale Expression analysis



Small-scale expression of shikimate kinase shows protein at approximately 20kDa. Hourly fractions T1 – T4 show increase in expression over time.

Large-scale Expression Analysis



Large-scale expression supports results from small-scale expression. Protein is expressed in both soluble form and in inclusion bodies.

Discussion/Conclusion

- Gene encoding shikimate kinase has been successfully cloned.
- Small and large-scale expression analyses show expression of shikimate kinase
- Protein is expressed in soluble form and in inclusion bodies.
- Shikimate kinase has been successfully purified (data not shown).

Future Work

- Steady-state kinetic characterization of enzyme
- Evaluate effect of marine sponge metabolites like hymenidine, avarone and derivatives on shikimate kinase

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