Structural & Functional Characterization of 3-Deoxy-D-*arabino*-Heptulosonate 7-Phosphate Synthase from *Helicobacter pylori* & *Mycobacterium tuberculosis*

Celia Jane Webby

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ABSTRACT

The shikimate pathway, responsible for the biosynthesis of aromatic compounds, is found in microorganisms and plants but absent in higher organisms. This makes the enzymes of this pathway attractive as targets for the development of antibiotics and herbicides. Recent gene disruption studies have shown that the operation of the shikimate pathway is essential for the viability of *M. tuberculosis*, validating the choice of enzymes from this pathway as targets for the development of novel anti-TB drugs.

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (DAH7PS) catalyzes the first committed step of the shikimate pathway. Two distinct classes of DAH7PS have been defined based on sequence similarity. The type I DAH7PSs are well characterized, however prior to this project there was limited mechanistic and no structural information about type II enzymes. Sequence identity between type I and type II enzymes is less than 10% raising the possibility that they represent distinct protein families, unrelated by evolution.

We have functionally characterized the type II enzyme from *Helicobacter pylori*, and have shown that type I and type II enzymes catalyze a metal-dependent ordered sequential reaction following the same stereochemical course. We have solved the structure of the type II DAH7PS from *M. tuberculosis* using single-wavelength anomalous diffraction (SAD) methods and the structure reveals a tightly associated dimer of $(\beta/\alpha)_8$ TIM barrels. The monomer fold, the arrangement of key residues in the active site, and the binding modes of PEP and Mn^{2+}, all match those of the type I enzymes. This similarity of protein fold and catalytic architecture makes it unequivocal that type I and type II enzymes are related by divergent evolution from a common ancestor. Interestingly, there are significant differences in the additional structural elements that extend from the core $(\beta/\alpha)_8$ barrel and in the quaternary structure. Further structural and functional analysis of *M. tuberculosis* DAH7PS revealed that the two major additions decorating the barrel are involved in the binding of the aromatic amino acids. Two distinct inhibitory binding sites for Trp and Phe have been identified providing an explanation for the synergistic inhibition displayed with Trp and Phe. The role of several active site residues of *Mt*-DAH7PS in enzyme catalysis has also been investigated.

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ABBREVIATIONS

AEC	Anion exchange chromatography
Amp	Ampicillin
A5P	D-Arabinose 5-phosphate
BTP	1,3-*bis*(tris(hydroxymethyl)methylamino)propane
CCP4	Collaborative Computational Project number 4
CEC	Cation exchange chromatogrpahy
Da	Dalton
DAH7P	3-deoxy-D-*arabino*-heptulosonate 7-phosphate
DNA	Deoxy-ribose nucleic Acid
dNTP	Deoxyribo nucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylenediamine tetra-acetic acid (di-sodium salt)
E4P	D-Erythrose 4-phosphate
FPLC	Fast protein liquid chromatography
G3P	D-Glyceraldehyde 3-phosphate
G6P	D-Glucose 6-phosphate
HCl	Hydrochloric acid
IEX	Ion exchange chromatography
IMAC	Immobilized metal affinity chromatography
IPTG	Isopropyl-1-thio-β-D-galactopyranoside
k_{cat}	Turnover number
KDO8P	3-Deoxy-D-*manno*-octulosonate 8-phosphate
K_2HPO_4	di-Potassium hydrogen orthophosphate
K_M	Michaelis constant
LB broth	Luria-Bertani broth
MW	Molecular weight
MWCO	Molecular weight cut-off
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
OD	Optical density
ORF	Open reading frame

PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PDB	Protein data bank
PEP	Phosphoenolpyruvate
Phe	Phenylalanine
pI	Isoelectric point
P_i	Inorganic phosphate
ppm	Parts per million
Psi	Pounds per square inch
R5P	D-Ribose 5-phosphate
2dR5P	2deoxyR5P
Rpm	Revolutions per minute
SAD	Single wavelength anomalous dispersion
SDS	Sodium dodecyl sulfate
SEC	Size exclusion chromatography
Se-Met	Selenomethionine
TCEP	Tris(2-carboxyethyl)phosphine hydrochloride
TEV	Tobacco etch virus
Thesit	Polyethyleneglycol dodecyl ether
TIM	Triose phosphate isomerase
Trp	Tryptophan
Tyr	Tyrosine
UV	Ultraviolet
V_{max}	Maximum reaction velocity

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