Biochemical Characterization of Metal-Dependent 3-Deoxy-D-*manno*-Octulosonate 8-Phosphate Synthases from *Chlorobium tepidum* & *Acidithiobacillus ferrooxidans*

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2007

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A thesis presented in partial fulfillment of the requirements for the degree of

Masterate of Science

in

Biochemistry

at Massey University, Turitea, Palmerston North

New Zealand

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ABSTRACT

3-Deoxy-D-*manno*-octulosonate 8-phosphate (KDO8P) synthase is the enzyme responsible for catalyzing the first reaction in the biosynthesis of KDO. KDO is an essential component in the cell wall of Gram-negative bacteria and plants. This compound is not present in mammals; therefore the enzymes responsible for its biosynthesis are potential targets for the development of new antibiotic agents. KDO8P synthase catalyzes the condensation reaction between phosphoenol pyruvate (PEP) and D-arabinose 5-phosphate (A5P) to form KDO8P.

Two types of KDO8P synthase have been identified; a metal-dependent type and a non metal-dependent type. KDO8P synthase from the organism *Chlorobium tepidum* (*Cte*) has been partially purified and partially characterized. In line with predictions based on sequence alone, the activity of this enzyme is dependent on the presence of a divalent metal ion and is sensitive to the presence of the metal chelating agent EDTA. *Cte* KDO8P synthase was found to have the highest activity in the presence of Mn^{2+} or Cd^{2+}.

KDO8P synthase from the organism *Acidithiobacillus ferrooxidans* (*Afe*) has also been cloned, purified and biochemically characterized. *Afe* KDO8P synthase was also found to be a metallo enzyme and the catalytic activity is highest in the presence of Mn^{2+} or Co^{2+}. *Afe* KDO8P synthase was found to exist as a tetramer in solution and is most active within the pH range of 6.8 to 7.5 and within a temperature range of 35 ºC to 40 ºC. Sequence analysis suggests that this enzyme has characteristics conserved throughout the metallo and the non-metallo KDO8P synthases and is closely related to the metal-dependent 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAH7P) synthases. The role of several active-site residues of *Afe* KDO8P synthase has been investigated. A C21N mutant of *Afe* KDO8P synthase was found to retain 0.5% of wild-type activity and did not require a divalent metal ion for catalytic activity. This suggests that the metallo and non-metallo KDO8P synthases have similar catalytic mechanisms.

ACKNOWLEDGEMENTS

Thank you to my supervisor Emily Parker for all the help and guidance and for encouraging me to pursue postgraduate studies. She has provided me with a great environment to learn and introduced me to some great people along the way. Also thanks for the coffee and lunch shouts. I will look forward to catching up with you over a wine or two in Lorne sometime in the future.

Thank you to my co-supervisor Geoff Jameson for helping with my work both in this project and throughout my time at Massey University. I wish I had some more exciting crystals for you to help me work with, but thanks for the help at the end and for the pretty pikkies!! Thank you also to Mark Patchett for providing the *C. tepidum* KDO8P synthase for me to work on. We couldn't have chosen a worse protein to try and purify, but thanks anyway!

Thanks to all the team in the EJP research group at Massey University. It has been great to be able to work with a vibrant group of students and researchers. Special thanks to Fiona Cochrane for looking over my shoulder for the first six months of my masters, showing me the way around a research lab, and giving me the odd Post-It note if I had done something wrong! And to Linley Schofield, two words – chocolate cake! I have learnt a lot from everybody in this group.

Thanks to my family for supporting me for five years at Massey University and for all the free meals when I was living off a student allowance! Thanks especially to my wife Monique who encouraged me to go and do what I wanted to do and for doing her best to stay out of my way during the last few weeks of writing up!

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ABBREVIATIONS

AEC	Anion exchange chromatography
Afe	*Acidithiobacillus ferrooxidans*
Amp	Ampicillin
ATP	Adenosine triphosphate
A5P	D-Arabinose 5-phosphate
BTP	1,3-*bis*(tris(hydroxymethyl)methylamino)propane
bp	Base pairs
CEC	Cation exchange chromatography
Cte	*Chlorobium tepidum*
Da	Dalton
DAH7P	3-deoxy-D-*arabino*-heptulosonate 7-phosphate
DNA	Deoxyribo nucleic acid
dNTP	Deoxyribo nucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra-acetic acid (di-sodium salt)
E4P	D-Erythrose 4-phosphate
FPLC	Fast protein liquid chromatography
HCl	Hydrochloric acid
HIC	Hydrophobic interaction chromatography
IEC	Ion exchange chromatography
IPTG	Isopropyl-1-thio-β-D-galactopyranoside
k_{cat}	Turnover number
KCl	Potassium chloride
KDO8P	3-deoxy-D-*manno*-octulosonate 8-phosphate
K_m	Michaelis constant
LB broth	Luria-Bertani broth
LPS	Lipopolysaccharide

MW	Molecular weight
MWCO	Molecular weight cut-off
NaCl	Sodium chloride
$(NH_4)_2SO_4$	Ammonium sulfate
NMR	Nuclear magnetic resonance
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PEG	Polyethyleneglycol
PEP	Phosphoenolpyruvate
pI	Isoelectric point
P_i	Inorganic phosphate
Psi	Pounds per square inch
SDS	Sodium dodecyl sulfate
SEC	Size exclusion chromatography
Thesit	Polyethyleneglycol dodecyl ether
UV	Ultra violet
V_{max}	Maximum reaction velocity