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

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

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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 °C to 40 °C. 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.
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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
<table>
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<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>MWCO</td>
<td>Molecular weight cut-off</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}</td>
<td>Ammonium sulfate</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethyleneglycol</td>
</tr>
<tr>
<td>PEP</td>
<td>Phosphoenolpyruvate</td>
</tr>
<tr>
<td>pI</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>P\textsubscript{i}</td>
<td>Inorganic phosphate</td>
</tr>
<tr>
<td>Psi</td>
<td>Pounds per square inch</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SEC</td>
<td>Size exclusion chromatography</td>
</tr>
<tr>
<td>Thesit</td>
<td>Polyethyleneglycol dodecyl ether</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>V\textsubscript{max}</td>
<td>Maximum reaction velocity</td>
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