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**Substrate Analogues As Mechanistic Probes
For 3-Deoxy-D-Arabino-Heptulosonate 7-
Phosphate Synthase And 3-Deoxy-D-Manno-
Octulosonate 8-Phosphate Synthase**

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Meekyung Ahn

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For Cem

ABSTRACT

Substrate analogues as mechanistic probes for 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase and 3-deoxy-D-*manno*-octulosonate 8-phosphate synthase

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (DAH7P synthase) catalyses the condensation reaction between phosphoenolpyruvate (PEP) and the four-carbon monosaccharide D-erythrose 4-phosphate (D-E4P). 3-Deoxy-D-*manno*-octulosonate 8-phosphate synthase (KDO8P synthase) catalyses a closely related reaction of PEP with the five-carbon monosaccharide D-arabinose 5-phosphate (D-A5P). These enzymes are two functionally unrelated enzymes that share many mechanistic and structural features.

D-Threose 4-phosphate (D-T4P), L-threose 4-phosphate (L-T4P), D-arabinose 5-phosphate (D-A5P), D-lyxose 5-phosphate (D-L5P), and L-xylose 5-phosphate (L-X5P) have been prepared synthetically or enzymatically to provide insights into aspects of metal requirement and substrate specificity. These compounds were different stereoisomers of natural substrates D-E4P and D-A5P. The results presented in this thesis show that D-T4P and L-T4P (C2 and C3 stereoisomers of D-E4P) are substrates for the DAH7P synthases from *E. coli* and *P. furiosus*. For *N. meningitidis* KDO8P synthase, natural substrate D-A5P and L-X5P (the C4 epimer of D-A5P) were substrates, whereas D-L5P, the C3 epimer of D-A5P, was not. These observations show that the configuration of the C2 and C3 hydroxyl groups is not important for DAH7P synthase reaction, but having the correct configuration at these positions is critical for KDO8P synthase.

The analysis of the interaction of D-T4P and L-T4P with DAH7P synthase, and D-A5P, D-R5P, and L-X5P reveals previously unrecognised mechanistic differences between the DAH7P synthase-catalysed reaction and that catalysed by the closely related enzyme, KDO8P synthase.

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SUBSTRATE ANALOGUES AS MECHANISTIC PROBES FOR 3-DEOXY-D-ARABINO-HEPTULOSONATE 7-PHOSPHATE SYNTHASE AND 3-DEOXY-D-MANNO-OCTULOSONATE 8-PHOSPHATE SYNTHASE

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Abbreviations

D-A5P	D-arabinose 5-phosphate
AHBA	3-amino 5-hydroxybenzoate
ATP	adenosine triphosphate
BSA	bovine serum albumin
BTE	boron trifluoride etherate
BTP	1,3-bis(tris(hydroxymethyl)-methylamino)propane
DAH7P	3-deoxy-D- <i>arabino</i> -heptulosonate 7-phosphate
D-DAO8P	3-deoxy-D- <i>altro</i> -octulosonate 8-phosphate
3-deoxyA5P	3-deoxy arabinose 5-phosphate
4-deoxyA5P	4-deoxy arabinose 5-phosphate
2-deoxyE4P	2-deoxy erythrose 4-phosphate
3-deoxyE4P	3-deoxy erythrose 4-phosphate
2-deoxyR5P	2-deoxy ribose 5-phosphate
L-DGO8P	3-deoxy-L- <i>gulo</i> -octulosonate 8-phosphate
D-DLH7P	3-deoxy-D- <i>lyxo</i> -heptulosonate 7-phosphate
L-DXH7P	3-deoxy-L- <i>xylo</i> -heptulosonate 7-phosphate
DHQ	3-dehydroquininate
DHS	3-dehydroshikimate
DPA	dipicolinic acid
DTNB	5,5'-dithio-bis(2-nitrobenzoate)
EDTA	ethylenediaminetetra acetic acid
EPSP	5-enolpyruvyl shikimate 3-phosphate
D-E4P	D-erythrose 4-phosphate
ESI	negative ion electrospray mass spectrometry
ESI-TOF MS	time-resolved electrospray ionisation mass spectrometry
EtOH	ethanol

D-F6P	D-fructose 6-phosphate
D-Gal6P	D-galactose 6-phosphate
G3P	D-glycerol 3-phosphate
G ⁻ bacteria	Gram negative bacteria
G ⁺ bacteria	Gram positive bacteria
D-Glu6P	D-glucose 6-phosphate
glyphosate	N-(phosphonomethyl)glycine isopropylamine salt
GMH	<i>L-glycero-D-manno</i> -heptose
homophosphonate	4,5-dideoxy-5-phosphono-D-erythro-pentose
iminoE4P	imino erythrose 4-phosphate
KDO	3-deoxy- <i>D-manno</i> -octulosonate acid
KDO8P	3-deoxy- <i>D-manno</i> -octulosonate 8-phosphate
K_M	Michaelis constant
K_i	inhibition constant
LAH	lithium aluminum hydride
LPS	lipopolysaccharide
NAD ⁺	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide reduced form
D-L5P	D-lyxose 5-phosphate
PABA	<i>para</i> -aminobenzoate
PEP	phosphoenolpyruvate
phosphonate	4-deoxy-4-phosphono-D-erythro-tetrose
P_i	inorganic phosphate
D-R5P	D-ribose 5-phosphate
D-T4P	D-threose 4-phosphate
L-T4P	L-threose 4-phosphate
S7P	sedoheptulose 7-phosphate
tlc	thin layer chromatography
UV	ultra-violet
THF	tetrahydrofuran
D-X5P	D-xylose 5-phosphate
L-X5P	L-xylose 5-phosphate

DAH7P synthase	3-deoxy-D- <i>arabino</i> -heptulosonate 7-phosphate synthase
DHQase	dehydroquinase
DHQS	3-dehydroquinase synthase
EPSP synthase	5-enolpyruvyl shikimate 3-phosphate synthase
G3P dehydrogenase	D-glycerol 3-phosphate dehydrogenase
KDO8P synthase	3-deoxy-D- <i>manno</i> -octulosonate 8-phosphate

<i>A. aeolicus</i>	<i>Aquifex aeolicus</i>
<i>A. pyrophilus</i>	<i>Aquifex pyrophilus</i>
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
<i>E. coli</i>	<i>Escherichia coli</i>
<i>H. pylori</i>	<i>Helicobacter pylori</i>
<i>M. tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
<i>P. furiosus</i>	<i>Pyrococcus furiosus</i>
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
<i>T. maritima</i>	<i>Thermotoga maritima</i>
<i>N. meningitidis</i>	<i>Neisseria meningitidis</i>

Publication

Parts of chapters two, four, and six in this thesis have been published.

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