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Synthetic targets as mechanistic probes for the key biosynthetic enzyme, dehydroquinate synthase

A dissertation submitted to
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by

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Para mis padres Ruth y Antonio
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Abstract

Dehydroquinate synthase (DHQS) catalyses the five-step transformation of the seven carbon sugar 3-deoxy-β-arabinopyranose 7-phosphate (DAH7P) to the carbacycle dehydroquinate (DHQ). Multiple studies have described in detail the mechanism of most of the steps carried out by DHQS with the exception of the final cyclisation step. In this study, (3S)-3-fluoro-DAH7P and (3R)-3-fluoro-DAH7P (fluorinated analogues of DAH7P) were produced and assayed across three phylogenetically distinct sources of DHQS in order to determine the role of the enzyme during the cyclisation step of the reaction.

Incubation of (3S)-3-fluoro-DAH7P with DHQS from Escherichia coli, Pyrococcus furiosus, and Kiwifruit resulted in the production of different ratios of (6S)-6-fluoro-DHQ and 1-epi-(6S)-6-fluoro-DHQ for each enzyme. In addition, enzyme catalysis showed a slowing of reaction rates when (3S)-3-fluoro-DAH7P was used, suggesting that the fluorine at C-3 is stabilising the enol pyranose. An increase in the stabilisation of the fluoro-enol pyranose would allow release of this substrate intermediate from the enzyme to compete with the on-going on-enzyme reaction.

The differences in the ratio of products formed suggest that the cyclisation occurs in part on the enzyme and that the epimeric product arises only by an abortive reaction pathway where the (3S)-3-fluoro-enol pyranose is prematurely released and allowed to cyclise free in solution. Once in solution, the (3S)-3-fluoro-enol pyranose could undergo a conformational change in the ring leading to the formation of the epimeric product. Furthermore, it is suspected that the position of fluorine influences the likely transition-state in carbacycle formation leading to the production of the epimeric product.
This research has illuminated the role of the enzyme in guiding the correct stereochemistry of the product and illustrates the important molecular interplay between the enzyme and substrate.
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Abbreviations

Bn  Benzyl
BTP  1,3-bis(tris(hydroxymethyl)amino)propane
DAH7P  3-deoxy-D-arabino-heptulosonate-7-phosphate
DAST  diethylaminosulfur trifluoride
DCM  dichloromethane
DHQ  dehydroquinate
DHQase  dehydroquinase
DHQS  dehydroquinase synthase
DMAP  4-dimethylaminopyridine
DMSO  dimethyl sulfoxide
DNA  deoxyribonucleic acid
DTT  dithiothreitol
E4P  erythrose-4-phosphate
E  extinction coefficient
EDTA  ethylenediaminetetraacetic acid disodium salt
ESMS  electrospray mass spectrometry
ESPS  5-enolpyruvyl-shikimate-3-phosphate
Et  ethyl
G-6-P  glucose-6-phosphate
IPTG  isopropylthio-β-D-galactoside
K_M  Michaelis constant
k_cat  catalytic constant
LB  Luria Bertani
Me  methyl
NAD^+  nicotinamide adenine dinucleotide
NaHMDS  sodium hexamethyldisilazide
NBS  N'-bromosuccinamide
NMR  nuclear magnetic resonance
NOE  nuclear Overhauser enhancement
OD_{600}  optical density at 600nm
PAGE  polyacrylamide gel electrophoresis
PEP  phosphoenol pyruvate
Ph  phenyl
P_i  inorganic phosphate
Ppm  parts per million
pTs  p-toluenesulfonic acid
Rt  room temperature
SDS  sodium dodecyl sulfate
TBAF  tetra-n-butylammonium fluoride
t-Bu  potassium tert-butoxide
THF  tetrahydrofuran
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMP</td>
<td>Trimethyl phosphite</td>
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<tr>
<td>UV</td>
<td>ultra violet</td>
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