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

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Leonardo Negrón

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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-D-*arabino*-heptulosonate 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, (3*S*)-3-fluoro-DAH7P and (3*R*)-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 (3*S*)-3-fluoro-DAH7P with DHQS from *Escherichia coli*, *Pyrococcus furiosus*, and Kiwifruit resulted in the production of different ratios of (6*S*)-6-fluoro-DHQ and 1-*epi*-(6*S*)-6-fluoro-DHQ for each enzyme. In addition, enzyme catalysis showed a slowing of reaction rates when (3*S*)-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 (3*S*)-3-fluoro-enol pyranose is prematurely released and allowed to cyclise free in solution. Once in solution, the (3*S*)-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.

Contents

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	dehydroquinate 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
*p*Ts	*p*-toluenesulfonic acid
Rt	room temperature
SDS	sodium dodecyl sulfate
TBAF	tetra-*n*-butylammonium fluoride
t-Bu	potassium *tert*-butoxide
THF	tetrahydrofuran

TLC	thin layer chromatography
TMP	Trimethyl phosphite
UV	ultra violet