Structural Characterization of 3-Dehydroquinate Synthase II

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Abstract

Aromatic amino acids tryptophan, tyrosine and phenylalanine are derived from a common precursor, chorismate, which is produced by the seven-step shikimate pathway in plants, fungi, *Bacteria* and *Archaea*. In *Archaea* the shikimate pathway typically begins with the alternative substrates, L-aspartate semialdehyde and 6-deoxy-5-ketofructose-1-phosphate, and so requires different enzymes to catalyse the first two steps in the pathway compared to those used in the (common) bacterial pathway. The archaeal enzyme for the second step, 3-dehydroquinate synthase type 2 (DHQS2), catalyses the oxidative deamination of 2-amino-3, 7-dideoxy-D-threo-heptoulsonic acid (formed in step 1) followed by cyclisation to produce 3-dehydroquinate, at which point the alternative and common shikimate pathways converge. No DHQS2 structures have yet been determined, and because DHQS2 enzymes have little sequence homology with their DHSQ1 analogues, they may have a novel fold. Bioinformatic methods were used to predict the solubility, stability and likelihood of sequenced DHQS2s to form crystals, and the five highest ranked were chosen for structural studies. *Methanococcus maripaludis, Desulfatibacillum alkenivorans*, *Methanospirillum hungatei*, and *Archaeoglobus veneficus* DHQS2 open reading frames were amplified by PCR and cloned into a modified pETite32a(+) vector in order to produce recombinant protein with an N-terminal, a C-terminal or no His_8-tag. Soluble recombinant DHQS2 proteins were produced in *Escherichia coli* DL41 (DE3), then purified by immobilized metal-ion affinity chromatography followed by size exclusion chromatography. C-terminally-tagged *M. maripaludis* DHQS2 with bound cofactor NAD^+ crystallised in two conditions: (i) 1.0 M ammonium sulfate, 0.1 M Bis-Tris pH 5.5 with 1% (w/v) PEG 3350; and (ii) 0.1 M CAPS at pH 10.5 with 40% (v/v) 2-methyl-2,4-pentanediol, but unfortunately the crystals were not of diffraction quality. Structure prediction using bioinformatic tools and/or far and near Circular Dichroism spectroscopy indicated that recombinant *M. maripaludis* DHQS2 was likely to have a secondary structure dominated by α-helices and had tertiary structure; recombinant *A. veneficus* was likely to have a secondary structure dominated by β-strands and had tertiary structure, while recombinant *D. alkenivorans*, and *M. hungatei* were more likely to assume a molten globule structure dominated by β-strands.

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Abbreviations

ADH	2-amino-3,7-dideoxy-D-threo-hept-6-ulsonic acid
ADHS	2-amino-3,7-dideoxy-D-threo-hept-6-ulsonic acid synthase
AmBic	Ammonium bicarbonate
AS	Anomalous scattering
ASA	L-Aspartate semi-aldehyde
BPB	Bromophenol blue
BSA	Bovine serum albumin
C-*Ave*DHQS2	C-terminally His_8-tagged *Archaeoglobus veneficus* 3-Dehydroquinate synthase type 2
CD	Circular dichroism
C-*Dal*DHQS2	C-terminally His_8-tagged *Desulfatibacillum alkenivorans* 3-Dehydroquinate synthase type 2
C-*Mhu*DHQS2	C-terminally His_8-tagged *Methanospirillum hungatei* 3-Dehydroquinate synthase type 2
C-*Mma*DHQS2	C-terminally His_8-tagged *Methanococcus maripaludis* 3-Dehydroquinate synthase type 2
C-*Mst*DHQS2	C-terminally His_8-tagged *Methanosphaera stadtmanae* 3-Dehydroquinate synthase type 2
Cryo-EM	Cryo-Electron Microscopy
C-*Saz*DHQS2	C-terminally His_8-tagged *Sulfurihydrogenibium azorense* 3-Dehydroquinate synthase type 2
C-score	Confidence score
DAH7P	3-Deoxy-D-arabino-heptulsonic acid 7-phosphate
DAH7PS	3-Deoxy-D-arabino-heptulsonic acid 7-phosphate synthase
DDH	3,7-Dideoxy-D-threo-hepto-2,6-diulosonic acid
DHQ	3-Dehydroquinate
DHQS	3-Dehydroquinate synthase
DHQS2	3-Dehydroquinate synthase type 2
DHS	Dehydroshikimate
DKFP	6-Deoxy-5-ketofructose 1-phosphate
DMSO	Dimethyl sulfoxide

DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotides
DTT	Dithiothreitol
E4P	Erythrose 4-phosphate
EP	Expert pool
EDTA	Ethylenediaminetraacetic acid
ESI	Electrospray ionization
F-1,6-P	Fructose-1, 6-phosphate
G6P	Glucose-6-phosphate
gDNA	Genomic DNA
HCl	Hydrochloric acid
His_8-tag	Eight histidine tag
HMMs	Hidden Markov models
HPAP	Hydroxypyruvaldehyde phosphate
IMAC	Immobilisation metal ion affinity chromatography
IOR	Indolpyruvate oxioreductase
IPTG	Isopropyl-β-D-thiogalactopyranoside
I-TASSER	Iterative threading assembly refinement
LB	Luria broth
MAD	Multi-wavelength anomalous dispersion
MCS	Multiple cloning site
MGS	Massey genome service
MIR	Multiple isomorphous replacement
ModpETite	Modified pETite vector
NAD^+	Nicotinamide adenine dinucleotide
N-*Ave*DHQS2	N-terminally His_8-tagged *Archaeoglobus veneficus* 3-Dehydroquinate synthase type 2
N-*Dal*DHQS2	N-terminally His_8-tagged *Desulfatibacillum alkenivorans* 3-Dehydroquinate synthase type 2
N-*Mhu*DHQS2	N-terminally His_8-tagged *Methanospirillum hungatei* 3-Dehydroquinate synthase type 2
N-*Mma*DHQS2	N-terminally His_8-tagged *Methanococcus maripaludis* 3-Dehydroquinate synthase type 2

NMR	Nuclear magnetic resonance
N-*Mst*DHQS2	N-terminally His_8-tagged *Methanosphaera stadtmanae* 3-Dehydroquinate synthase type 2
N-*Saz*DHQS2	N-terminally His_8-tagged *Sulfurihydrogenibium azorense* 3-Dehydroquinate synthase type 2
OD_{600}	Optical density at 600 nm
PABA	*p*-aminobenzoic acid
PCR	Polymerase chain reaction
PDB	Protein data bank
PEP	Phosphoenol pyruvate
PONDR	Predictor of natural disordered regions
SCRs	Structurally conserved regions
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
Se-Met	Seleno-methionine
SOB	Super optimal broth
TCEP	Tris-(2-carboxyethyl)phosphine
TEV	Tobacco etch virus
T_m	Melting temperature
VRs	Variable regions

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