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Crystal structure of a type II dehydroquinase dehydratase-like protein from *Bifidobacterium longum*

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Abstract

Dehydroquinase dehydratase (DHQD) catalyzes the third step in the biosynthetic shikimate pathway. Here we identify a *Bifidobacterium longum* protein with high sequence homology to type II DHQDs but no detectable DHQD activity under standard assay conditions. A crystal structure reveals that the *B. longum* protein adopts a DHQD-like tertiary structure but a distinct quaternary state. Apparently forming a dimer, the *B. longum* protein lacks the active site aspartic acid contributed from a neighboring protomer in the type II DHQD dodecamer. Relating to the absence of protein–protein interactions established in the type II DHQD dodecameric assembly, substantial conformational changes distinguish the would-be active site of the *B. longum* protein. As *B. longum* possess no other genes with homology to known DHQDs, these findings imply a unique DHQD activity within *B. longum*.

Keywords

Post-translational activation; Quaternary structure; Shikimate pathway; X-ray crystal structure; Structural genomics

Introduction

The shikimate pathway comprises seven enzymes that catalyze the biosynthesis of chorismate, a precursor for a number of important metabolites, including the aromatic amino acids [1]. The shikimate pathway enzymes are essential in plants and in a number of pathogenic microorganisms but lack mammalian counterparts, and, therefore, should represent reasonable targets for the development of herbicides and antimicrobials [4, 21].

Dehydroquinate dehydratase (DHQD) reversibly catalyzes the conversion of dehydroquinate to dehydroshikimate, the third step in the shikimate pathway. Within bacteria, two mechanistically dissimilar DHQD types exhibit distinct phylogenetic distributions [7, 9]. Type I DHQDs contain an active site lysine that forms a Schiff base with the substrate [2], which acts as an electron sink to facilitate the catalytic dehydration [11, 12, 25]. Type II DHQDs act via an unrelated reaction mechanism, characterized by an enolate reaction intermediate [8, 20, 23].

Crystal structures of type II DHQDs from 8 organisms have been deposited to the Protein Data Bank (PDB) [7, 10, 15, 23]. These structures unanimously reveal a dodecameric functional assembly, established by a tetramer of triangular trimers (Fig. 1a). With this quaternary layout, each protomer in the dodecamer establishes distinct intra- (threefold) and inter- (twofold) trimer interfaces (Fig. 1a). The threefold interface seems to be particularly important for function, as here interacting protomers jointly contribute active site residues.

The Gram-positive *Bifidobacterium longum* possess a single annotated DHQD. This protein shares high sequence homology with type II DHQDs and all of the key active site residues are conserved (Fig. 2). We show here that, lacking DHQD activity, this protein behaves unlike characterized type II DHQDs. Clarifying the basis of inactivity, a crystal structure reveals that the *B. longum* protein forms a dimer that lacks a critical active site residue contributed from the neighboring protomer in the type II DHQD dodecamer.

Materials and methods

Gene cloning and protein expression and purification

Utilizing previously described protocols [5, 17, 24], the annotated *B. longum* aroQ gene (UniProt/TrEMBL ID, B3DSD7_BIFLD) was sub-cloned into the pMCSG7 vector and expressed in BL21-CodonPlus (DE3)-RIL cells (Stratagene). Following expression, cells were harvested by centrifugation and resuspended in a buffer containing 10 mM tris (pH 8.3), 500 mM NaCl, 10 % glycerol, and 5 mM β -mercaptoethanol. Cells were sonicated and the soluble fraction of the resulting cell lysate passed over a 5 ml HisTrapFF column. Protein was step-eluted from the column with 0.5 M imidazole and passed over a HiPrep 26/60 gel-filtration column. A single peak collected from the size exclusion column was incubated overnight at 4 °C with hexa-histidine tagged tobacco etch virus protease (to remove the expression tag) and passed over the HisTrapFF column, this time with the flow-through being collected.

Protein crystallization and data collection

Sitting drop crystal screens were performed at room temperature, with 1 μ l of 8.4 mg ml⁻¹ protein being added to 1 μ l of reservoir. A crystal grown in a condition containing 0.1 MMIB (2:3:3 of sodium malonate, imidazole, and boric acid) buffer (pH 5) and 25 % PEG 1500 was transferred to its reservoir solution for cryoprotection and cryopreserved in liquid nitrogen. Diffraction data were collected at station D of the Life Sciences Collaborative Access Team at the Advance Photon Source, Argonne, Illinois.

Structure determination and refinement

Data were indexed, integrated, and scaled in HKL-3000 [19]. The crystal structure was solved by molecular replacement using Phaser [16] and the *Mycobacterium tuberculosis* type II DHQD structure (PDB code 2DHQ) as a search model. The structure was iteratively refined in Refmac [18] and manually corrected in Coot [6] based on electron density maps. The final atomic coordinates and structure factors are available in the PDB under the accession code 3U80.

Dehydroquinase (DHQD) activity assay

As previously described [12, 13], the formation of the conjugated enone carboxylate in the DHQD product dehydroshikimate was spectroscopically monitored by measuring an increase in absorbance ($\lambda = 234$, $\epsilon = 12 \text{ mM}^{-1} \text{ cm}^{-1}$) [3].

Results

Analysis of the recombinantly expressed *B. longum* protein by size exclusion chromatography and multiangle light scattering revealed a dimeric solution state (data not shown). As the oligomeric assembly appears to be critical for type II DHQD function, the protein was tested for DHQD activity. Unlike type I and type II DHQD positive controls, assays failed to detect any DHQD activity of the *B. longum* protein (data not shown).

To address its unique oligomeric state and apparent lack of DHQD activity, a 1.60 Å resolution crystal structure of the *B. longum* DHQD-like protein was determined by molecular replacement (Table 1). Corresponding to the presumed dimeric state, two protomers are present within the crystallographic asymmetric unit (Fig. 1b). Structural alignments of the final refined structure reveal that the two protomers are similar in tertiary structure to each other (RMSD = 0.37 Å over 93 Cα atoms) and to previously described type II DHQDs (RMSD = 0.75 Å over 79 Cα atoms, to the *M. tuberculosis* DHQD, PDB code 2DHQ).

The presumed dimer interface (established by the two protomers within the asymmetric unit of the DHQD-like structure) is formed by the C-terminal α-helix 4 and the extension of the 5-stranded beta-sheet of each protomer and buries ~ 1,235 Å² (Fig. 1b). Inspection of symmetry related molecules failed to identify a higher oligomeric arrangement. Structural alignments using the dimer reveal conservation of the type II DHQD twofold interface within the crystal (Fig. 1c). The threefold interface, however, is notably absent from the structure. The absence of this interface very likely relates to the lack of DHQD activity. The insertion of Asp87 from a neighboring protomer in the trimer into the active site, where its side chain hydrogen bonds with the reaction product, is conspicuously absent in this *B. longum* DHQD-like structural assembly (Fig. 1d). Moreover, major conformational changes distinguish most of the remaining residues that would form the DHQD active site. Two regions that contribute to the threefold interface within the type II DHQD structure exhibit distinct conformational states in the *B. longum* DHQD-like structure. Gly13–Gly24 is disordered within both protomers in the crystallographic asymmetric unit. Pro75–Ser82, which connects β-strand 3 to α-helix 3 (Fig. 1d), is shifted from its conformation in DHQD. In addition, Asp111–Arg116, which is not at the threefold interface but interacts with Pro75–Ser82 in the DHQD structures, is disordered in both DHQD-like protomers (Fig. 1d).

In light of its unique structure, the *B. longum* DHQD-like sequence was reanalyzed to see if a simple signature that might account for the significant differences in tertiary and quaternary structure could be identified. A sequence alignment revealed that a single residue strictly conserved across the PDB's 8 dodecameric type II DHQDs differs in the *B. longum*

protein (Asp111 is substituted for a Glu in the other type II DHQDs) (Fig. 2). Given its conservative nature and localization at the protein surface, this substitution seems unlikely to be the source of the unique structural features of the DHQD-like protein. An analysis of the residues that would form the threefold interface revealed the identity of each is conserved in at least one of the other dodecameric DHQDs. Together, these observations argue against a single point mutation accounting for the distinct structural features exhibited by the *B. longum* protein.

A 2–3 amino acid insertion (from Met91 to Ile94) stands out as the one clear feature that distinguishes the *B. longum* DHQD-like primary sequence from that of the 8 other DHQDs (Fig. 2). The Met91–Ile94 containing α 3-helix is slightly shifted relative to its position in the *M. tuberculosis* structure (Fig. 1d). As the α 3-helix leads directly into the more drastically shifted Pro75–Ser82 loop, it is possible that this small insertion induces a subtle shift in the position of the α 3-helix, which, in turn, ripples across tertiary and, ultimately, quaternary structures. Additional studies will be necessary to ascertain whether Met91–Ile94 or some other aspect of primary structure is responsible for the unique tertiary and quaternary features of the *B. longum* protein.

Discussion

It is possible that the *B. longum* protein lost DHQD activity over evolutionary history while acquiring an alternative function. However, a BLAST search of the *B. longum* genome turned up no other genes with significant sequence homology to either of the known DHQD types. In the absence of an alternative DHQD candidate, it is possible that a post-translational modification or protein–protein interaction is required to activate DHQD functionality of the DHQD-like protein.

Interestingly, *B. longum* possess an unusual gene that encodes a protein predicted to contain both dehydroquinase synthase and the shikimate kinase activities on a single polypeptide chain (UniProt/TrEMBL ID, B3DSD8_BIFLD). Within the shikimate pathway, dehydroquinase synthase is one step upstream and shikimate kinase two steps downstream from DHQD. Given the intervening steps that connect its two activities, the functional advantage of a bifunctional dehydroquinase synthase/shikimate kinase fusion protein is unclear.

A precedent exists for the aggregation of shikimate pathway activities into a larger multifunctional complex. Within some fungal and protozoan species, activities for the five intermediate steps in the pathway are embedded in a single polypeptide chain [14, 22]. It is intriguing to speculate that the bifunctional dehydroquinase synthase/shikimate kinase protein might associate with the enzymes that catalyze the reaction steps that link its two activities, establishing a multi-step assembly functionally similar to the penta-functional organization exhibited in some eukaryotes. If this is the case, conformational changes induced by the formation of such a heteromultimer might be required for the catalytic activation of the *B. longum* DHQD-like protein. While requiring experimental validation, this proposal reconciles the apparent inactivity of the only candidate DHQD within the *B. longum* genome with the enigmatic organization of downstream and upstream steps in the shikimate pathway.

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Abbreviations

ASU	Asymmetric unit
DHQD	Dehydroquinase dehydratase
PDB	Protein Data Bank
RMSD	Root-mean-square-deviation

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Fig. 1.

Crystal structure of the DHQD-like protein from *B. longum*. **a** The *M. tuberculosis* type II DHQD (PDB code 2DHQ) dodecamer (a tetramer of trimers) is shown in surface representation. The three protomers that make up one trimer are colored *beige*, *orange*, and *yellow*. Two additional trimers visible in this perspective are colored *gray* and *green*. **b** The two protomers within the *B. longum* DHQDlike crystallographic asymmetric unit are colored *green* and *cyan*. **c** Superposition of the two protomers within the *B. longum* DHQD-like asymmetric unit on two trimers from the *M. tuberculosis* DHQD dodecamer. Each protomer within the *M. tuberculosis* trimers is colored a different shade of *gray*. The overlaid protomers are depicted in ribbon and the non-overlaid protomers in surface representation. Arrows denote the *M. tuberculosis* DHQD twofold (which coincides with the *B. longum* DHQD-like dimer interface) and threefold interfaces. **d** A superposition of protomer A of the *B. longum* DHQDlike (*cyan*) to the *M. tuberculosis* type II DHQD reaction product bound complex (PDB code 2DHQ, *brown*) is shown in stereo. Active site residues within the *M. tuberculosis* enzyme are represented as *sticks*, with corresponding residues in the *B. longum* protein numbered (several are disordered and thus not shown). The reaction product, dehydroshikimate (DHS), within the *M. tuberculosis* structure is colored *yellow* and the aspartic acid contributed by a neighboring molecule within the type II trimer is colored *pink*.

Fig. 2.

Sequence alignment of the DHQD-like protein from *B. longum* to 4 dodecameric type II DHQDs. The sequence alignment was prepared using ClustalW2. The *B. longum* DHQD-like secondary structure elements are depicted above the alignment. Residues that are conservatively substituted (red lettering, white background) or strictly conserved (white lettering, red background) are boxed. Type II DHQD active site residues are marked below the alignment by orange circles. Note that the aspartic acid (Asp87) that contributes to the active site of a neighboring protomer in the DHQD dodecamer is conserved in the *B. longum* enzyme

Table 1

X-ray data and refinement statistics

	DHQD-like
Crystal parameters	
Space group	P 2 ₁ 2 ₁ 2 ₁
Cell dimensions	
a, b, c (Å)	44.0, 72.7, 81.7
α, β, γ (°)	90, 90, 90
Data collection	
Wavelength (Å)	0.97900
Resolution (Å)	30–1.60 (1.64–1.60)
R _{sym} (%)	4.4 (50.3)
No. of unique reflections	35,461
Mean redundancy	7.3 (7.3)
Overall completeness (%)	99.9 (100)
Mean I/σ	40.8 (6.3)
Refinement residuals	
R _{free} (%)	20.3 (26.6)
R _{work} (%)	17.6 (22.7)
Completeness (%)	99.8 (98.2)
Model quality	
RMSD bond lengths (Å)	0.010
RMSD bond angles (°)	1.3
Molprobit Ramachandran distribution	
Most favored (%)	98.9
Allowed (%)	1.1
Disallowed (%)	0.0
Mean main chain B-factor (Å ²)	23.5
Mean overall B-factor (Å ²)	25.9
Mean solvent B-factor (Å ²)	37.5
Model contents	
Protomers in ASU	2
No. of protein atoms	1,302
No. of water molecules	168