Crystal structure of fuculose aldolase from the Antarctic psychrophilic yeast *Glaciozyma antarctica* PI12

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Fuculose-1-phosphate aldolase (FucA) catalyses the reversible cleavage of L-fuculose 1-phosphate to dihydroxyacetone phosphate (DHAP) and L-lactaldehyde. This enzyme from mesophiles and thermophiles has been extensively studied; however, there is no report on this enzyme from a psychrophile. In this study, the gene encoding FucA from *Glaciozyma antarctica* PI12 (GaFucA) was cloned and the enzyme was overexpressed in *Escherichia coli*, purified and crystallized. The tetrameric structure of GaFucA was determined to 1.34 Å resolution. The overall architecture of GaFucA and its catalytically essential histidine triad are highly conserved among other fuculose aldolases. Comparisons of structural features between GaFucA and its mesophilic and thermophilic homologues revealed that the enzyme has typical psychrophilic attributes, indicated by the presence of a high number of nonpolar residues at the surface and a lower number of arginine residues.

1. Introduction

Psychrophilic organisms thrive in cold environments (Morita, 1975) and are capable of growing at temperatures ranging from 20°C to below 0°C, with an optimum growth temperature near 15°C (D'Amico *et al.*, 2006). In order to survive at such low temperatures, psychrophiles incorporate an array of adaptive features. One of the most obvious is that their entire enzymatic complement must incorporate adaptations allowing them to function at low temperatures (Feller, 2013).

There are multiple environmental factors that psychrophilic enzymes must adapt to. Enzymatic activity generally decreases with lowered temperature, but the increase in the viscosity of the aqueous environment also lowers reaction rates (Gianese *et al.*, 2001). In comparison to their mesophilic counterparts, structural differences stemming from changed amino-acid compositions in cold-adapted enzymes have been reported (Kahlke & Thorvaldsen, 2012). In general, psychrophilic enzymes are known to be flexible and to have lower thermostability and high specific activity at low temperatures compared with their mesophilic counterparts. Various studies have been conducted to elucidate the factors that contribute to these features. Psychrophilic enzymes tend to have small

Table 1
Fuculose aldolases (not including variants) from different microorganisms deposited in the PDB.

Microorganism	PDB code	Reference
Mesophilic		
<i>Aquifex aeolicus</i> VF5 (AaFucA)	2irp	RIKEN Structural Genomics/Proteomics Initiative (unpublished work)
<i>Streptococcus pneumoniae</i> (SpFucA)	4c24, 4c25	Higgins <i>et al.</i> (2014)
<i>Bacteroides thetaiotaomicron</i> (BtFucA)	2opi	Midwest Center for Structural Genomics (unpublished work)
<i>Escherichia coli</i> (EcFucA)	1fua, 2fua, 3fua, 4fua	Dreyer & Schulz (1996), Joerger <i>et al.</i> (2000)
<i>Pseudomonas syringae</i> (PsFucA)	3ocr	Midwest Center for Structural Genomics (unpublished work)
Thermophilic		
<i>Thermus thermophilus</i> (TtFucA)	2ffl, 2fk5	Jeyakanthan <i>et al.</i> (2005)

Table 2
GaFucA production information.

Source organism	<i>G. antarctica</i>
DNA source	<i>G. antarctica</i> cDNA
Forward primer	5'-GACGACGACAAGATGAACCTCTGCCGCC-3'
Reverse primer	5'-GAGGAGAAGCCCGGCTACTTCTGTAC-3'
Cloning vector	pGEM-T
Expression vector	pET-30 Ek/LIC
Expression host	<i>E. coli</i> BL21 (DE3)
Complete amino-acid sequence of the construct produced	MNSAASVRNNALEEPVAGKGFDFGSGGLAALTGP-PTFATVEAERAWLKERLVAAIRIFANEGFDHT-VAGHLTVRDPENKHHFVWNPFGLAFLMTVSD-LILVNQEGTVIGGGKEGRRIVNLAFGMIHSAI-HKARPEVQAICHSHSTYGKAFSSLGKPLAITT-QDSCAFYGDVALGDFGGVVIEEKESGTIAVAL-QQKKAILLQNHGLLTVGTTIDSAVAFWIMLEK-QCQVQLLADAAGQTIPIDEPQAFTFKELGHE-QAGYFQASPYFQVIEHLQGEERYK

amino acids in the loop region, an increased number of hydrophobic residues on the solvent-exposed area, fewer hydrophobic residues in the enzyme core and a lower number of arginine and proline residues (De Maayer *et al.*, 2014).

Glaciozyma antarctica (formerly known as *Leucosporidium antarcticum*) is a basidiomycetous, obligate psychrophilic yeast habitant of Antarctica (Turchetti *et al.*, 2011). *G. antarctica* strain PI12 was isolated from sea ice near the Casey Research Station; it has an optimum growth temperature of 12°C but can grow at up to 20°C (Boo *et al.*, 2013). Whole-genome sequencing revealed 7857 protein-encoding genes (Mohd Firdaus Raih *et al.*, unpublished work). Several cold-active enzymes from *G. antarctica* have been reported, including α -amylase (AmyPI12; Ramli *et al.*, 2013) and chitinase (CHII; Ramli *et al.*, 2011); however, computer modelling was used to generate all of these enzyme structures. To better understand the cold-adaptive features of psychrophilic enzymes within *G. antarctica*, we decided to crystallize and determine the structure of one of its enzymes, fuculose-1-phosphate aldolase (GaFucA).

Fuculose-1-phosphate aldolase (EC 4.2.1.17) is a lyase within the glycolysis metabolic pathway that catalyses the reversible cleavage of L-fuculose-1-phosphate into dihydroxyacetone phosphate (DHAP) and L-lactaldehyde. This reaction is an essential step in L-fucose metabolism in microorganisms (Ghalambor & Heath, 1962). DHAP-dependent aldolases are enantioselective catalysts (Ozaki *et al.*, 1990) that can be used in the synthesis of rare sugars and related compounds that are often difficult to synthesize chemically (Joerger *et al.*, 2000). Fuculose-1-phosphate aldolase is also used in the synthesis of

iminocyclitols, which are inhibitors of glycosidases and glycosyltransferases that have therapeutic potential for a wide range of diseases (Fessner & Jennewein, 2006). Currently, 23 structures of FucA, including variants (generated by amino-acid substitution), have been deposited in the PDB from six different mesophilic and thermophilic microorganisms (Table 1).

This study reports a high-resolution crystal structure of GaFucA that enables comparison of the psychrophilic enzyme with its mesophilic and thermophilic counterparts, revealing the presence of a high number of nonpolar residues at the surface and a low number of arginine residues. In addition, the structure of GaFucA may provide an initial template for the development of new industrial and biotechnological applications.

2. Materials and methods

2.1. Macromolecule production

2.1.1. cDNA amplification and construction of pGaFucA. A cDNA encoding fuculose-1-phosphate aldolase was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using *G. antarctica* total RNA as the template. The primers used for amplification were 5'-CA AGA ATG AAC TCT GCC GCC-3' and 5'-CCC CTA CTT CCT GTA CTC-3'. The amplified fragment was inserted into a cloning vector, transformed into *Escherichia coli* DH5 α cells and plated onto a Luria-Bertani (LB) agar plate containing ampicillin (100 μ g ml⁻¹). The inserted sequence was verified as fuculose-1-phosphate aldolase by sequencing. The gene was then subcloned into the pET-30 Ek/LIC vector (Novagen) using the coding and reverse complement primer sequences. The resulting plasmid was sequenced to ensure fidelity and subsequently transformed into *E. coli* BL21 (DE3) cells (Novagen). GaFucA production is summarized in Table 2.

2.1.2. Protein expression and purification. A single transformant colony was inoculated into 50 ml LB medium supplemented with 50 μ g ml⁻¹ kanamycin and grown overnight at 310 K in a shaking incubator. These cultures were then used to inoculate 800 ml LB medium (containing 50 μ g ml⁻¹ kanamycin) and grown at 310 K until the optical density at 600 nm reached 0.5–0.7; 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) was then added to induce expression of the protein. After further growth at 289 K for 18 h, the cells were harvested by centrifugation at 4000g for

Table 3

Crystallization.

Method	Hanging drop
Plate type	24-well Linbro plate
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	11
Buffer composition of protein solution	20 mM Tris-HCl pH 8.0, 50 mM NaCl
Composition of reservoir solution	0.1 M Tris pH 8.0, 25% PEG 8000, 0.25 M MgCl ₂
Volume and ratio of drop	1 µl protein solution:1 µl reservoir solution
Volume of reservoir (µl)	500

Table 4

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	MX2, Australian Synchrotron
Wavelength (Å)	0.9537
Temperature (K)	100
Detector	ADSC Quantum 315 CCD
Crystal-to-detector distance (mm)	200
Rotation range per image (°)	0.5
Total rotation range (°)	180
Exposure time per image (s)	2
Space group	<i>P</i> 4 ₂ 1 ₂
<i>a</i> , <i>b</i> , <i>c</i> (Å)	84.33, 84.33, 78.50
α , β , γ (°)	90.0, 90.0, 90.0
Mosaicity (°)	0.41
Resolution range (Å)	37.71–1.34 (1.41–1.34)
Total No. of reflections	971734 (80657)
No. of unique reflections	61957 (7472)
Completeness (%)	96.6
Multiplicity	15.7 (10.8)
$\langle I/\sigma(I) \rangle$	14.6 (2.0)
Overall <i>B</i> factor from Wilson plot (Å ²)	14.6

30 min at 277 K and stored at 193 K. The frozen bacterial pellet was thawed and resuspended in buffer *A* (20 mM Tris-HCl pH 8.0, 300 mM NaCl, 20 mM imidazole). The resuspended cells were disrupted by sonication and the cell lysate was clarified by centrifugation (15 300g for 30 min) and filtered to remove cell debris and fine particles. Since the recombinant protein contained an N-terminal His tag, it was passed through a column pre-packed with Ni-NTA agarose (Qiagen). Proteins were eluted from the column with buffer *A* supplemented with 300 mM imidazole and subsequently applied onto a HiTrap Q FF column (GE Healthcare Life Sciences) which was equilibrated in buffer *B* (20 mM Tris-HCl pH 8.0). GaFucA was eluted in a linear NaCl gradient from 0 mM to 1 M over 60 ml. Fractions containing GaFucA were pooled, concentrated to approximately 5 ml by ultrafiltration (Vivaspin, 10 kDa cutoff; Sartorius AG) and loaded onto a HiLoad 16/60 Superdex 200 column (GE Healthcare Life Sciences) pre-equilibrated with 20 mM Tris pH 8.0, 300 mM NaCl. The sample purity and molecular weight were assessed by denaturing polyacrylamide gel electrophoresis (SDS-PAGE; Laemmli, 1970). Fractions that contained pure protein were pooled and concentrated using ultrafiltration (Vivaspin, 10 kDa cutoff; Sartorius AG). Buffer exchange was then carried out to store the protein in 20 mM Tris-HCl pH 8.0, 50 mM NaCl. The GaFucA concentration was calculated to be 34 mg ml⁻¹, with an extinction coefficient of 24 075 M⁻¹ cm⁻¹ as determined with the *ProtParam* tool (<http://web.expasy.org/>)

Table 5

Structure solution and refinement.

Values in parentheses are for the outer shell.

Resolution range (Å)	37.71–1.34 (1.41–1.34)
Completeness (%)	96.6
σ Cutoff	1.34
No. of reflections, working set	58682 (2043)
No. of reflections, test set	3132 (103)
Final <i>R</i> _{cryst}	0.171
Final <i>R</i> _{free}	0.186
No. of non-H atoms	
Protein	1985
Ion	0
Ligand	1
Water	232
Total	2218
R.m.s. deviations	
Bonds (Å)	0.006
Angles (°)	1.063
Average <i>B</i> factors (Å ²)	
Overall	19.0
Protein	1990
Ion	0
Ligand	1
Water	232
Ramachandran plot	
Most favoured (%)	98
Allowed (%)	2

protParam) using an ND-1000 spectrophotometer (Thermo Scientific). The protein was filtered to remove impurities and stored at 277 K for use in crystallization trials.

2.2. Crystallization

GaFucA crystals were grown at 293 K using the hanging-drop vapour-diffusion method. Initial crystallization screening was performed using the commercially available kit The JCSG+ Suite (Qiagen). Each drop consisted of 1 µl protein solution at a concentration of 11 mg ml⁻¹ and 1 µl reservoir solution, and the mixture was allowed to equilibrate against 500 µl reservoir solution. Small crystals appeared on equilibration against four conditions: (i) 0.1 M Tris pH 8.5, 1.26 M ammonium sulfate, 0.2 M Li₂SO₄, (ii) 0.1 M HEPES pH 7.5, 0.6 M Na₂HPO₄, 0.6 M K₂HPO₄, (iii) 0.1 M Tris pH 8.0, 20% PEG 8000, 0.2 M MgCl₂ and (iv) 0.1 M Na/KPO₄ pH 6.2, 50%

Figure 1

Crystal of *G. antarctica* fucose aldolase grown using the optimized crystallization technique. Crystals grew to a volume of between 100 and 200 µm³ after optimization.

PEG 200. Protein, salt, precipitant concentration and pH were varied to improve the crystal size and quality. Crystals grew to a volume of between 100 and 200 μm^3 (Fig. 1) within 2–7 d after optimization and those suitable for X-ray diffraction were collected, transferred into a cryoprotectant solution consisting of the mother liquor and 23%(v/v) glycerol and flash-cooled in liquid nitrogen in a mounted cryoloop. Crystallization information is summarized in Table 3.

2.3. Data collection and processing

A complete X-ray data set was collected from a single cryopreserved crystal on the MX2 beamline at the Australian

Synchrotron. Data were processed, integrated and scaled using *iMosflm* (Battye *et al.*, 2011) and various programs from the *CCP4* suite (v.6.4.0; Winn *et al.*, 2011). The final data-collection and processing statistics are summarized in Table 4.

2.4. Structure solution and refinement

Phases were obtained by molecular replacement using fuculose aldolase (PDB entry 3ocr; Midwest Center for Structural Genomics, unpublished work) from *Pseudomonas syringae* (PsFucA) as the search model using *Phaser* (McCoy, 2007). The *PHENIX* package (Adams *et al.*, 2010) was used to improve the initial structure. Iterative rounds of model

Figure 2 Multiple sequence alignment of fuculose aldolases from *G. antarctica* (PDB entry 4xxf), *P. syringae* (PDB entry 3ocr), *B. thetaiotaomicron* (PDB entry 2opi), *A. aeolicus* (PDB entry 2irp), *T. thermophilus* (PDB entry 2flf), *E. coli* (PDB entry 2fua) and *S. pneumoniae* (PDB entry 4c24). Residues that are strictly conserved (white letters on a red background) and conservatively substituted (black letters on a yellow background) are boxed. Histidine-triad residues are marked below the alignment with blue stars. One of the histidine residues for PDB entry 2opi has been replaced by a glutamine, whereas the other fuculose aldolases have conserved histidine-triad residues.

building were then carried out, alternating between manual building within *Coot* (Emsley *et al.*, 2010) and automatic refinement within *PHENIX* (Adams *et al.*, 2010). Water molecules were automatically added during the later rounds of refinement. Atomic coordinates and diffraction data for the

final GaFucA structure have been deposited in the Protein Data Bank with accession code 4xxf. Statistics of data collection and refinement are summarized in Table 5.

2.5. Structure analysis

Multiple sequence alignment using *Clustal Omega* (Sievers *et al.*, 2011) and *ESPript* (<http://esprict.ibcp.fr>; Robert & Gouet, 2014) was carried out between the GaFucA sequence and those of other fucose aldolases. For comparisons with psychrophilic, mesophilic and thermophilic enzymes, the sequence identity and final structure of GaFucA were compared with those of PsFucA and *Thermus thermophilus* FucA (TtFucA) using *PDBFold* v.2.59. Glycine, proline and arginine contents were determined using the *ProtParam* tool (<http://web.expasy.org/protparam>; Gasteiger *et al.*, 2005). Hydrophobic interactions were measured by *PDBEPIA* (Krissinel & Henrick, 2007). Hydrogen bonds and polar and nonpolar accessible surface areas were calculated with the online software *VADAR* (<http://vadar.wishartlab.com/>; Willard *et al.*, 2003) using a probe radius of 1.4 Å. Structural figures were generated using *PyMOL* (v.1.3; Schrödinger).

3. Results

3.1. GaFucA amino-acid composition

The open reading frame of *Gafuca* encoded a protein consisting of 285 amino-acid residues with a predicted molecular weight of 30 688 Da and a pI of 5.99. A multiple sequence alignment of GaFucA with other FucAs was performed using *Clustal Omega* (Sievers *et al.*, 2011) and *ESPript* (Robert & Gouet, 2014; Fig. 2). The catalytically important histidine triad is highly conserved among all of these aldolases. Gly88, Gly106, His127, Leu208 and Glu226 were also conserved among fucose aldolases.

3.2. Structural analyses of GaFucA

G. antarctica fucose aldolase was successfully cloned *via* ligation-independent cloning and the expression of recombinant GaFucA was maximal at 289 K. The protein was purified

Figure 3
(a) SEC profile of recombinant GaFucA. Arrows indicate the elution volumes of proteins of known molecular weight (labelled in kDa). (b) SDS-PAGE analysis of recombinant GaFucA (Lanes 2–5). Markers (Lane 1) are indicated on the left in kDa.

Figure 4
Diffraction data for a GaFucA crystal. The outer black circle corresponds to a resolution of 1.34 Å.

by a three-step isolation protocol involving immobilized metal-affinity chromatography (IMAC), anion-exchange chromatography and size-exclusion chromatography (SEC). Combined, these methods yielded a nearly pure protein of 285 amino acids with a six-His tag at the N-terminus. The deduced molecular weight for this enzyme is approximately 30 kDa. The major elution volume peak in SEC corresponds to a tetramer in solution with an approximate molecular weight of 120 kDa and the minor elution volume peak corresponded to a monomer in solution (30 kDa) (Fig. 3*a*). The purified protein was analysed by SDS-PAGE (Fig. 3*b*).

Conditions amenable for crystallization of GaFucA were determined by setting up the protein against a broad array of conditions. A complete diffraction data set was collected from

crystals grown in 0.1 M Tris pH 8.0, 25% PEG 8000, 0.25 M MgCl₂ (Fig. 4). Crystallographic analysis indicated that the crystal diffracted to 1.34 Å resolution and belonged to space group *P*4₂1₂, with unit-cell parameters *a* = 84.33, *b* = 84.33, *c* = 78.50 Å, $\alpha = \beta = \gamma = 90.0^\circ$. The data set was processed and scaled, and the data-collection statistics are presented in Table 4. The Matthews coefficient indicated that each asymmetric unit contained a single monomer of GaFucA, corresponding to a solvent content of 50.3% and an acceptable *V_M* of around 2.48 Å³ Da⁻¹ (Matthews, 1968). The physiological homotetramer is thus created by the crystallographic fourfold symmetry axis.

Structure determination was carried out by molecular replacement using the known aldolase structure from *P. syringae* (PsFucA; PDB entry 3ocr; Midwest Structural Genomics Consortium, unpublished work). The final GaFucA model consists of residues 26–283, comprising nine α -helices and an eight-stranded mixed β -sheet (Fig. 5). As illustrated in Fig. 5, the β -strands (S1–S8) that define the enzymatic core are sandwiched between α -helices (H1–H9). These structural features are similar to those of other class II fucose aldolases (Dreyer & Schulz, 1996; Joerger *et al.*, 2000; Marchler-Bauer *et al.*, 2013). Most of the backbone and side-chain atoms of the structure have well defined electron density, except for residues 1–25 and loop 178–186 connecting secondary-structural elements S6 and H7, which are disordered and could not be fitted to our satisfaction. Alternate conformations were observed for the side chains of Trp46, His70, Gln138, Gln196, Gln248, His258 and Glu279. An analysis of the Zn²⁺-ion binding site in the GaFucA structure showed that the three metal-liganding residues (His142, His144 and His206) adopt a catalytically competent conformation and OMIT maps indicate clear electron density for the presence of a bound metal ion at this site (Fig. 6). According to Laitaoja *et al.* (2013), this His–His–His triad motif is an important characteristic of lyases that allows efficient catalysis.

Figure 5
The three-dimensional structure of GaFucA contains nine α -helices (H1–H9) and eight β -strands (S1–S8).

Figure 6
The zinc ion (grey sphere) is bound by the catalytic triad (His142, His144 and His206) of GaFucA.

Table 6

Comparison of structural features between three FucAs.

	GaFucA	PsFucA	TtFucA
pI	5.99	6.09	6.72
Amino acids	283	273	200
Glycine (%)	9.5	9.2	9.5
Proline (%)	3.5	4.0	5.0
Arginine (%)	3.5	6.2	8.0
Salt bridges	11	10	9
Hydrogen bonds	281	281	181
Hydrophobic interactions	249	251	189
Exposed surface area (Å ²)			
Total	11522.4	12102.2	9536.9
Polar residues	2138.4	2132.5	1431.7
Nonpolar residues	7330.4	7143.7	5679.0

3.3. Overall structural comparison of psychrophilic, mesophilic and thermophilic fucose aldolases

GaFucA has a low sequence identity to PsFucA (31%) and TtFucA (21%). Superposition of the GaFucA structure with those of PsFucA and TtFucA yielded r.m.s.d.s of 1.656 Å based on 222 C α atoms and 1.849 Å based on 171 C α atoms, respectively. However, overall comparison of the three-dimensional structures of GaFucA, PsFucA and TtFucA revealed that all structures have a similar architecture, whereby GaFucA shares 83% and 72% identity with PsFucA and TtFucA, respectively (Fig. 7).

3.4. Comparison of the structural features of psychrophilic, mesophilic and thermophilic FucA

To reveal the molecular basis of the cold temperature adaptation of fucose aldolase, several structural features were compared between GaFucA, PsFucA and TtFucA (Table 6). A combination of structural features, including the numbers of glycines, prolines and arginines, ion pairs, hydrogen bonds and hydrophobic interactions and the solvent-accessible area, are thought to affect structural flexibility in psychrophilic enzymes (De Maayer *et al.*, 2014).

Arginine, glycine and proline are believed to be the signature amino acids for psychrophilic enzymes. The differences in the quantities of these amino acids have been reported for several cold-adapted enzymes with respect to their mesophilic

and thermophilic counterparts (Feller, 2013; De Maayer *et al.*, 2014). Arginine plays a significant role in thermal adaptation as its side chain can form up to five hydrogen bonds and the guanidium group provides arginine with the ability to form more than one salt bridge. Thus, psychrophilic enzymes tend to have a lower arginine content (Mrabet *et al.*, 1992; D'Amico *et al.*, 2001). We observed that GaFucA had the lowest percentage (3.5%) of arginine compared with PsFucA (6.2%) and TtFucA (8.0%).

An increased number of glycine residues is thought to increase the flexibility of the backbone (Kim *et al.*, 1999) and hence is a characteristic of psychrophilic enzymes. A high number of proline residues, on the other hand, is believed to increase protein stability (Pack & Yoo, 2004), and thus psychrophilic proteins have a reduced proline content. However, only small differences in glycine and proline content were observed in the three FucA structures.

In addition, the numbers of salt bridges and hydrogen bonds were also calculated. We observed that GaFucA and PsFucA have a similar number of salt bridges (11 and ten, respectively) and the same number of hydrogen bonds (281), whilst in contrast GaFucA and TtFucA have larger differences in the numbers of salt bridges and hydrogen bonds (nine and 181, respectively, in TtFucA).

The exposed surface areas for polar and nonpolar residues of GaFucA, PsFucA and TtFucA were calculated and analysed. GaFucA has the largest nonpolar accessible surface area, while the thermophilic TtFucA has the smallest, suggesting that GaFucA possesses cold-adaptive features.

4. Discussion

Psychrophilic enzymes are characterized by their higher flexibility and activity at low temperature compared with their mesophilic and thermophilic counterparts. Factors including an increased number of hydrophobic surface residues, extended surface loops, decreased interdomain and subunit ion, electrostatic and aromatic interactions, reduced proline and arginine content, and increased glycine content are considered to be important in stabilizing psychrophilic enzymes at cold temperatures (De Maayer *et al.*, 2014). In this

Figure 7

Structures of (a) psychrophilic FucA (from *G. antarctica*), (b) mesophilic FucA (from *P. syringae*) and (c) thermophilic FucA (from *T. thermophilus*). The structural architecture of FucA is very similar in the three microorganisms.

study, we have determined the structure of fuculose aldolase from the psychrophilic yeast *G. antarctica* in order to expand our knowledge of psychrophilic adaptation. We compared the structural features of GaFucA with those of FucA from *P. syringae* (mesophilic) and *T. thermophilus* (thermophilic).

GaFucA has a similar overall structural architecture to its mesophilic (PsFucA) and thermophilic (TtFucA) counterparts. A search of the PDB revealed that other FucAs also possess similar structures consisting of β -sheets sandwiched by α -helices, with the active site (histidine triad) highly conserved and bound by a ligand via a divalent ion (Dreyer & Schulz, 1996; Jeyakanthan *et al.*, 2005; Karthik *et al.*, 2013; Krissinel & Henrick, 2007). GaFucA was found to have a greater exposed surface area comprising of nonpolar residues and this observation correlates with the psychrophilic enzymes adenylate kinase from *Bacillus globisporus* (Bae & Phillips, 2004) and α -amylase from *G. antarctica* (Ramli *et al.*, 2013). Hydrophobic (nonpolar) residues are often found on the protein surface of psychrophilic enzymes and this results in a higher acidic pI compared with their mesophilic and thermophilic counterparts (Jahandideh *et al.*, 2007; Struvay & Feller, 2012). This surface hydrophobicity has been implicated to improve protein flexibility owing to the reduced entropy of water molecules at low temperature and is one of the strategies for the environmental adaptation of psychrophilic enzymes (Siddiqui *et al.*, 2006). In addition, GaFucA had a lower percentage of arginine (approximately twofold lower) when compared with PsFucA and TtFucA. This corresponds to the work of Kim *et al.* (1999), who found that the arginine content of malate dehydrogenase from *Aquaspirillum arcticum* is lower than that of its thermophilic counterpart. Although we observed the lowest percentage of arginine in GaFucA, the total numbers of hydrogen bonds and salt bridges were similar to those in PsFucA. This is likely to be owing to the effect of other residues contributing to hydrogen bonds/salt bridges in the protein molecule, as GaFucA has a total of 283 residues compared with 273 in PsFucA. We also suggest that the positions of arginine substitutions in GaFucA compared with PsFucA and TtFucA contribute to its psychrophilic attributes. Besides these, no significant trends were detected. Thus, our observation indicates that GaFucA may employ a different strategy to function in cold temperature by adopting specific combinations of structural features. Each enzyme adopts its own strategy and not all mechanisms are applicable for psychrophilic enzymes to survive in a cold environment (Feller & Gerday, 2003; De Maayer *et al.*, 2014).

5. Conclusions

The decreased number of arginine residues and the large nonpolar exposed surface area may contribute towards the cold-adaptive features of *G. antarctica* FucA. The GaFucA model could be used to unravel the structure–function relationships of this enzyme and may contribute to a better understanding of other psychrophilic enzymes. Additional studies focused in this direction are now in progress.

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