Crystal structure of a β -aminopeptidase from an Australian *Burkholderia* sp.

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Received 12 April 2017

Accepted 24 May 2017

Edited by W. N. Hunter, University of Dundee, Scotland

Keywords: BcA5-BapA; β -aminopeptidases; crystallization; *Burkholderia* sp.; β -amino acids; Ntn hydrolases.

PDB reference: BcA5-bapA, 5tzb

Supporting information: this article has supporting information at journals.iucr.org/f

β -Aminopeptidases are a unique group of enzymes that have the unusual capability to hydrolyze N-terminal β -amino acids from synthetic β -peptides. β -Peptides can form secondary structures mimicking α -peptide-like structures that are resistant to degradation by most known proteases and peptidases. These characteristics of β -peptides give them great potential as peptidomimetics. Here, the X-ray crystal structure of BcA5-BapA, a β -aminopeptidase from a Gram-negative *Burkholderia* sp. that was isolated from activated sludge from a wastewater-treatment plant in Australia, is reported. The crystal structure of BcA5-BapA was determined to a resolution of 2.0 Å and showed a tetrameric assembly typical of the β -aminopeptidases. Each monomer consists of an α -subunit (residues 1–238) and a β -subunit (residues 239–367). Comparison of the structure of BcA5-BapA with those of other known β -aminopeptidases shows a highly conserved structure and suggests a similar proteolytic mechanism of action.

1. Introduction

β -Aminopeptidases are a unique group of enzymes that have the unusual capability to hydrolyze N-terminal β -amino acids from synthetic β -peptides (Geueke & Kohler, 2007; Fuchs *et al.*, 2011; Komeda & Asano, 2005; Heck *et al.*, 2006). β -Peptides are resistant to degradation by most known proteases and peptidases, and can form secondary structures mimicking α -peptide-like structures (Frackenpohl *et al.*, 2001; Seebach *et al.*, 1996; Wiegand *et al.*, 2002; Gademann *et al.*, 1999; Gademann & Seebach, 2001; Hook *et al.*, 2005; Webb *et al.*, 2005). These properties make β -peptides useful as peptidomimetics (Gademann *et al.*, 2001). Bioinformatic analyses have identified β -aminopeptidases in a wide range of prokaryotes and eukaryotes; however, their biological role(s) remain unknown (Geueke *et al.*, 2006; Fuchs *et al.*, 2011; Merz *et al.*, 2012).

The β -aminopeptidases belong to the L-aminopeptidase D-Ala-esterase/amidase (DmpA)-like family of the N-terminal nucleophile (Ntn) hydrolases (Bompard-Gilles *et al.*, 2000; Oinonen & Rouvinen, 2000). Ntn hydrolases are produced as inactive polypeptide precursors that are activated after self-cleavage to form both α and β subunits. Within the α subunits, the substrate-binding regions determine the specificity of the β -aminopeptidases. The β subunits contain a conserved catalytic triad consisting of Ser-Ser-Glu/Asp that acts to both self-process and catalyze substrate conversion (Merz *et al.*, 2012).

To date, five bacterial β -aminopeptidases have been isolated and functionally characterized (Fanuel, Thamm *et al.*, 1999; Heck *et al.*, 2006; Geueke *et al.*, 2005, 2006; Komeda &

Table 1

Macromolecule-production information.

Source organism	<i>Burkholderia</i> sp., denoted BcA5
DNA source	Overexpression construct from <i>E. coli</i> BL21(DE3)pLysS
Forward primer	5'-GAATTGCCATATGCGCAGGAGGATCTCGG-3'
Reverse primer	5'-CGCTTGTGACTCAGGCGCGGCC-3'
Cloning vector	pET-28a(+)
Expression vector	pET-28a(+)
Expression host	<i>E. coli</i> BL21(DE3)pLysS
Complete amino-acid sequence of the construct produced	MGSSHHHHHSSGLVPRGSHM ₁ RTRDLGIRIGLG-TPGRFNAITDVPGRVGHCTLNEENGASIRT-GVTVIEPRAGAAHDSPCFAGVHVLNGNDATG-LEWIREAGLLTTPAIYTNTHSVGAVRDALVAN-EREAAAGRVYWCMPVVMETDGLLNDIWGQHVSAAHVQRALAAQTPVAEGGVGGGTGMICHE-FKGGIGTASRVLAADAGGWTGALVQANYGVR-EMLRVAGYPVGEVLRHVPSPFRAPDAQGEAGM-GSIVVTIATDAPLLPHQCTRLAQRASVGLARV-GGGTDESSEDIFLAFATGNDGLPAANYGSKGA-PTTGVMVNDHISALFVAAAEAVEEAIVNAL-VAGGDVESRGARVEGLGQARLLDALREVWGRP-GRGA

Asano, 2005; Komeda *et al.*, 2004; Fuchs *et al.*, 2011; Gueke & Kohler, 2007). Like many aminopeptidases, each of the characterized enzymes has a substrate preference based on the N-terminal β -amino acids. The structures of three β -aminopeptidases have been determined (PDB entries 1b65, 3n2w and 2drh; Bompard-Gilles *et al.*, 2000; Merz *et al.*, 2012; RIKEN Structural Genomics/Proteomics Initiative, unpublished work), all of which show a conserved tetrameric arrangement. In comparison to other Ntn hydrolases such as penicillin acylase, the direction and the connectivity of the secondary-structure elements in the monomer is unique to the DmpA-like family of Ntn hydrolases (Bompard-Gilles *et al.*, 2000; McVey *et al.*, 2001; Suresh *et al.*, 1999).

In this study, we report the X-ray crystal structure of a β -aminopeptidase from a Gram-negative *Burkholderia* sp. isolated from activated sludge from a wastewater-treatment plant in Australia. In our study, only the genus of this strain was determined and the species could not be identified; therefore, based on structural similarity to previously characterized β -aminopeptidases, we designated it BcA5-BapA. The data allowed comparative analysis with other structurally known β -aminopeptidases and indicate that BcA5-BapA may have a similar substrate specificity to that of DmpA from *Ochrobactrum anthropi*.

2. Materials and methods

2.1. Expression and purification of BcA5-BapA

BcA5-BapA was amplified from the genomic DNA of the isolated *Burkholderia* sp. and cloned into pCR-Blunt II-TOPO Zero Blunt vector. The gene was then cloned into the pET-28a(+) expression vector. For protein expression, *E. coli* BL21(DE3)pLysS cells were transformed with the pET-28a(+) expression vector containing His₆-tagged BcA5-BapA. The expression strain was grown in 500 ml 2 $\times$ YT broth supplemented with 50 μ g ml⁻¹ kanamycin and 34 μ g ml⁻¹

Table 2

Crystallization.

Method	Sitting-drop vapour diffusion (screening), hanging-drop vapour diffusion (production)
Plate type	INTELLI-PLATE 96-3 (Art Robbins Instruments) (screening), 24-well XRL Plate (Molecular Dimensions) (production)
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	15 (screening), 7.5 (production)
Buffer composition of protein solution	50 mM Tris-HCl pH 8.0, 150 mM NaCl
Composition of reservoir solution	35% 2-methyl-2,4-pentanediol, 0.1 M sodium cacodylate pH 7.0, 5% PEG 8000
Volume and ratio of drop	200 nl (screening), 2 μ l (production); 1:1
Volume of reservoir (μ l)	50 (screening), 500 (production)

chloramphenicol at 30°C with shaking until an OD₆₀₀ of 0.55 was reached. Gene expression was induced by the addition of 2 mM isopropyl β -D-1-thiogalactopyranoside and the culture was incubated for a further 4 h at 30°C with shaking. Protein purification was achieved by resuspension of the cell pellet in 50 mM Tris, 150 mM NaCl pH 8 before lysis using an Avestin cell disruptor. The whole cell lysate was clarified by centrifugation. Imidazole was added to the soluble fraction of the retained supernatant to a final concentration of 5 mM. The protein was purified using TALON Superflow resin. After loading the crude lysate, the resin was washed with 4 $\times$ 20 ml 50 mM Tris-HCl, 150 mM NaCl, 5 mM imidazole pH 8.0. The bound proteins were eluted with a stepwise imidazole gradient (20, 60, 100 and 200 mM). Recombinant BcA5-BapA was further purified by size-exclusion chromatography using a Superdex 200 10/300 column equilibrated with 50 mM Tris-HCl, 150 mM NaCl pH 8.0. The purity of the protein was assessed by SDS-PAGE and the protein concentration was determined using the Pierce BCA protein assay. Macromolecule-production information is summarized in Table 1.

2.2. Crystallization

BcA5-BapA was concentrated to 15 mg ml⁻¹ for initial sparse-matrix screening. The Monash Macromolecular Crystallization Facility was utilized to set up sitting-drop sparse-matrix screens (INTELLI-PLATE 96-3, Art Robbins Instruments). Crystals were observed in 11 different conditions. Three conditions were selected for optimization and screening by the hanging-drop method: (i) 35–45% 2-methyl-2,4-pentanediol, 0.1 M sodium cacodylate pH 6–7.5, 5% PEG 8000, (ii) 15–20% PEG 600, 0.01 M zinc chloride, 0.1 M Tris pH 7.5–9.0 and (iii) 0.05–0.2 M potassium thiocyanate, 25–35% PEG MME 2000. The cryoprotective properties of 2-methyl-2,4-pentanediol led us to focus on this condition for further optimization. Final crystals of BcA5-BapA were obtained with a protein concentration of 7.5 mg ml⁻¹ in 35% 2-methyl-2,4-pentanediol, 0.1 M sodium cacodylate pH 7.0, 5% PEG 8000. Crystallization conditions are summarized in Table 2.

Table 3

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	MX2 beamline, AS
Wavelength (Å)	0.95369
Temperature (K)	100
Detector	ADSC Quantum 201r CCD
Crystal-to-detector distance (mm)	200
Rotation range per image (°)	0.2
Total rotation range (°)	0–360
Exposure time per image (s)	0.5
Space group	$P2_1$
a, b, c (Å)	91.45, 133.26, 117.07
α, β, γ (°)	90, 91.6, 90
Mosaicity (°)	0.73
Resolution range (Å)	62.71–1.98 (2.01–1.98)
Total No. of reflections	787571 (31912)
No. of unique reflections	190171 (8372)
Completeness (%)	97.4 (87.1)
Multiplicity	4.1 (3.8)
$\langle I/\sigma(I) \rangle$	8.1 (1.4)†
R_{merge}	0.140 (0.924)
$R_{\text{p.i.m.}}$	0.079 (0.538)
$CC_{1/2}$	0.986 (0.591)
Overall B factor from Wilson plot (Å ²)	19.73

† The resolution at which the mean $I/\sigma(I)$ falls below 2.0 is 2.03 Å; however, the $CC_{1/2}$ and completeness indicate that a resolution of 1.98 Å is appropriate for the data.

2.3. Data collection and processing

A single BcA5-BapA crystal was harvested (CryoLoop, Hampton Research) and flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K using synchrotron radiation on the MX2 beamline (3BM1) at the Australian Synchrotron (AS). Data were indexed and integrated using *XDS* (Kabsch, 2010) and scaled and averaged in *AIMLESS* (Evans & Murshudov, 2013) from the *CCP4* suite (Winn *et al.*, 2011). Data-collection and processing statistics are detailed in Table 3.

2.4. Structure refinement and analysis

Initial phases were solved by molecular replacement using *Phaser* (McCoy *et al.*, 2005) with chain A of PDB entry 1b65 as a search model (Bompard-Gilles *et al.*, 2000). Eight clear peaks in both the rotation and translation functions were evident, and these packed well within the asymmetric unit. The asymmetric unit had a solvent content of 49.70%. Model building and refinement were performed using *PHENIX* (Adams *et al.*, 2010) and *Coot* (Emsley & Cowtan, 2004). The final refinement statistics are shown in Table 4. The coordinates and structure factors are available from the Protein Data Bank (PDB entry 5tzb). Analysis of the protein–protein interface was performed using *PDBEPIA* (Krissinel & Henrick, 2007). All crystal structure figures were prepared and analyses were performed in *PyMOL* v.1.3r2 (Schrödinger). Structure and sequence comparisons were carried out using *PDBFold* (Krissinel & Henrick, 2004), *PDBEPIA* (Krissinel & Henrick, 2007), *Clustal Omega* (McWilliam *et al.*, 2013; Sievers *et al.*, 2011) and *LALIGN* from ExPASy (Li *et al.*, 2015; McWilliam *et al.*, 2013).

Table 4

Structure refinement.

Values in parentheses are for the outer shell.

R_{work}	0.1979 (0.2580)
R_{free}	0.2380 (0.2951)
No. of non-H atoms	
Total	22172
Macromolecules	20386
Ions (Ca ²⁺)	4
Water	1782
No. of protein residues	2834
R.m.s.d., bond lengths (Å)	0.007
R.m.s.d., angles (°)	1.06
Ramachandran favoured (%)	97
Ramachandran outliers (%)	0.04
Clashscore	2.5 [99th percentile†]
Average B factor (Å ²)	
Overall	28.5
Macromolecules	28.1
Ligands	22.6
Solvent	33.0
Calculated DPI (Å) using R_{free} (23.8%)	0.166

† *MolProbity*; $N = 714$, 1.98 ± 0.25 Å.

3. Results

3.1. Amino-acid composition of BcA5-BapA

Putative β -aminopeptidases have been found in bacteria, fungi, archaea and plants; however, to date only five have been isolated and characterized (Rawlings *et al.*, 2014; Geueke & Kohler, 2007; Merz *et al.*, 2012; Fuchs *et al.*, 2011). Of these characterized enzymes, the structures of three have been elucidated. These structures are of DmpA from *O. anthropi* (PDB entry 1b65; Bompard-Gilles *et al.*, 2000), 3-2W4 BapA from *Sphingosinicella xenopeptidilytica* (PDB entry 3n2w; Merz *et al.*, 2012) and PH0078 from *Pyrococcus horikoshii* OT3 (PDB entry 2drh; RIKEN Structural Genomics/Proteomics Initiative, unpublished work). The remaining two functionally characterized β -aminopeptidases, BapF from *Pseudomonas aeruginosa* PAO1 and Ps BapA from *Pseudomonas* sp. MCI3434, both hydrolyse β -alanyl substrates (Fuchs *et al.*, 2011; Komeda & Asano, 2005). The enzyme described here, BcA5-BapA, is 367 amino acids in length with a predicted molecular weight of 37.9 kDa. BcA5-BapA shares 40–70% amino-acid sequence similarity with the other β -aminopeptidases and is most closely related to BapF and Ps BapA (71 and 75% identity, respectively). Alignment of the BcA5-BapA sequence with those of the other β -aminopeptidases shows that the residues of BcA5-BapA important for self-processing and catalysis are conserved in the other family members (Supplementary Fig. S1). The conserved catalytic residues in BcA5-BapA are Ser239, Ser277 and Asp279.

3.2. Overall structure of BcA5-BapA

BcA5-BapA was successfully cloned, expressed and purified via a two-step isolation protocol. BcA5-BapA, like all Ntn hydrolases (Bompard-Gilles *et al.*, 2000; Merz *et al.*, 2012; Brannigan *et al.*, 1995), is produced as a single polypeptide precursor that undergoes self-cleavage between residues Gly238 and Ser239 to form the active enzyme. The active form

of the BcA5-BapA enzyme is therefore composed of α and β subunits. The major elution peak from size-exclusion chromatography (Supplementary Fig. S2a) corresponds to a tetramer in solution, as has been observed for other β -aminopeptidases in the family. SDS-PAGE analysis of this species (Supplementary Fig. S2b) showed that the tetramer was composed of both the cleaved and uncleaved forms of the enzyme, indicating that self-cleavage had not proceeded to completion. Fractions containing the tetrameric species were pooled and concentrated for crystallization.

Crystallization conditions were determined by sparse-matrix screening and optimized in 24-well format. A complete diffraction data set to 1.98 Å resolution was collected from a large single crystal; the crystal belonged to space group $P2_1$, with unit-cell parameters $a = 91.45$, $b = 133.26$, $c = 117.07$ Å, $\beta = 90^\circ$ (Supplementary Fig. S2c). Structure solution was carried out by molecular replacement using chain A of DmpA (PDB entry 1b65; Bompard-Gilles *et al.*, 2000) as a search model. The asymmetric unit contained eight molecules of BcA5-BapA arranged as two distinct tetramers. Pairwise superposition of the eight chains (A–H) using chain A as a reference showed a highly conserved structure (the r.m.s.d. for all superpositions was <0.25 Å).

BcA5-BapA consists of residues 1–367 and the self-cleavage position is between Gly238 and Ser239. Therefore, the α subunit consists of residues 1–238 and the β subunit includes residues 239–367 (Fig. 1). The α -subunit residues 226–238, which precede the proteolytic cleavage site (Ser239) in BcA5-

BapA, were not visible in the electron density and are therefore likely to retain a high level of flexibility within the crystal. The overall structure of the BcA5-BapA monomer showed the characteristic four-layered $\alpha\beta\beta\alpha$ architecture, in which two β -sheets (formed by strands 1–4 and 5–9) are sandwiched between two pairs of α -helices (four in total) (Fig. 1).

Two complete tetramers of BcA5-BapA were observed in the asymmetric unit. The monomeric subunits are organized in a dihedral (D_2) symmetry, producing a well packed globular tetramer (Fig. 2). Overall, the tetrameric association of the subunits results in a total buried surface area of 17 580 Å² and a solvent-accessible surface area of 39 120 Å². The intermolecular interaction interface that mediates tetramerization involves a total of 31% of the total surface area of each monomer, and involves contacts along the molecular x axis (1789 Å²), y axis (1417 Å²) and z axis (1188 Å²). This extensive interface includes numerous hydrogen bonds (32 between monomers A and B and 27 between monomers C and D) and salt bridges (12 between monomers C and B and 13 between monomers D and A), all of which contribute a calculated free energy of dissociation (ΔG^{diss}) of 58 kcal mol^{−1}. Given the extensive network of interactions that mediate tetramerization, we suggest that the tetramer is a functional unit.

The functional tetramer has four active sites where self-processing and catalysis occur. This tetrameric association also forms the substrate-binding channel that is at the interface with three monomeric units, presenting a clear groove and

Figure 1

Cartoon model of the BcA5-BapA monomer. A cartoon diagram showing the secondary-structural elements of the BcA5-BapA monomer with the α subunit shown in magenta and the β subunit in cyan. The last residue of the α subunit, Phe226, is shown in stick representation and labelled. The first residue of the β subunit, Ser239, is shown in stick representation and labelled. Active-site residues are shown in stick representation and indicated in the boxed enlargement.

access to the active sites (Fig. 2). For example, the active site of chain *A* has a substrate-binding channel formed by residues in chains *B* and *D* (Fig. 2). This substrate-binding channel measures ~ 39 Å in length and spans both sides of the active-site entrance. The channel is lined with mainly hydrophobic residues. Two residues, Tyr129 in chain *A* and Trp120 in chain *D*, effectively reduce the access to the active site by forming stacking interactions that block the catalytic nucleophile from bulk solvent.

There are four active sites in the tetramer, each of which is located at the intermolecular interfaces between monomers (Fig. 2). The catalytic residues that constitute the active site in each monomer are Glu127 in the α subunit and Ser239, Ser277 and Asp279 in the β subunit (Fig. 1). The Ser239 residue is at the N-terminus of the self-processed β subunit and serves as the nucleophile in the catalytic mechanism. Residues Tyr129 and Asn201 are ideally positioned to stabilize negatively charged substrate–enzyme intermediates during catalysis. Glu127 is in the correct position to act as a proposed negative charge to ensure correct substrate positioning for attack by Ser239 (Oinonen & Rouvinen, 2000).

3.3. Comparative analysis of BcA5-BapA

Superposition of the BcA5-BapA tetramer with those of its homologues DmpA, PH0078 and 3-2W4 BapA shows a highly conserved quaternary structure, with each exploiting the tetrameric arrangement to provide substrate-binding pockets for the active sites. The superposition of chain *A* from each structure shows that all four structures adopt the same topology, with an overall r.m.s.d. of <1.5 Å. Despite the conserved overall topology, there are distinct differences

between each of the enzymes. The first of these is observed in the region just prior to the self-processing cleavage site (residues Gly238–Ser239). In this region, BcA5-BapA and PH0078 possess no secondary-structure elements, whereas 3-2W4 BapA contains a small two-stranded β -sheet insertion and DmpA has a short helical turn (Fig. 3). However, the major source of structural variation between the four homologues is residues 112–120 (BcA5-BapA numbering; Fig. 3). This region is thought to confer the substrate specificity of the enzyme. The most divergent of the homologues, 3-2W4 BapA, forms a twisted helical structure, possibly owing to the presence of a proline residue in the helix. DmpA has a helical turn, whereas PH0078 and BcA5-BapA simply have a loop structure to form the substrate pocket. BcA5-BapA also had an unstructured or loop region at residues 293–302 that is longer than in the other homologues and would likely result in a shallower or closed substrate-binding channel (Fig. 3). The result of these differences is a variation in the size of the

Figure 2

Surface image of the BcA5-BapA tetramer. The biological unit is a tetramer and each chain is indicated in a different colour. The residues that form the substrate-binding channel are coloured grey and the opening of the channel is also indicated by an arrow and labelled. The active-site residues are coloured black and are indicated by an arrow.

Figure 3

Comparison of the structures of the β -aminopeptidases. The cartoon model of BcA5-BapA is shown in magenta, DmpA (PDB entry 1b65) in light blue, PH0078 (PDB entry 2drh) in light green and 3-2W4 BapA (PDB entry 3n2w) in wheat. The first residue of the β -subunit is shown as black spheres and is indicated by a black arrow and BcA5-BapA numbering. The most variable region of the superposition is shaded in yellow and involves a region predominantly involved in substrate entry. Phe226, the last residue of the α subunit of BcA5-BapA, is shown in stick representation in this shaded region. The substrate-binding channel residues 112 and 120 in BcA5-BapA are indicated by black arrows and labelled. The equivalent residues to Trp120 are shown in stick representation in their respective colours. The position of the unique proline residue of 3-2W4 BapA (Pro119) is indicated in wheat. The extended loop structure formed by BcA5-BapA residues 293–302 are shown in stick representation and the residues are numbered where possible.

substrate-binding channels. By measuring the distance from the nucleophilic Ser to the 'gatekeeper' residue of each channel (Trp120 in BcA5-BapA, Arg126 in 3-2W4 BapA, Trp139 in DmpA and Ser128 in PH0078; Fig. 3), we estimated the width of the substrate channels as 12.3 Å in 3-2W4 BapA, 8.6 Å in PH0078, 6.3 Å in DmpA and 5.5 Å in BcA5-BapA. The 3-2W4 BapA homologue clearly has the widest substrate-binding channel, which is predicted to allow the access of larger substrates (Merz *et al.*, 2012).

4. Discussion

In this study, we report the crystal structure of BcA5-BapA, a β -aminopeptidase from *Burkholderia* sp. BcA5-BapA was self-processed into α and β subunits but purified as a soluble tetramer. Single crystals were produced and the X-ray crystal structure was solved to a resolution of 2.0 Å. The structure of BcA5-BapA showed the typical $\alpha\beta\beta\alpha$ sandwich fold that is common to all β -aminopeptidases. Superpositions of both the sequence and the structure of BcA5-BapA with those of other β -aminopeptidases show that the enzyme is highly conserved and supports the hypothesis that BcA5-BapA is a functional β -aminopeptidase that uses a similar mechanism of action. Based on this, we propose that Ser239 is a likely candidate for the nucleophilic residue and identified Glu127, Ser277 and Asp279 as putative active-site residues. Similar to its homologues, a substrate-binding pocket is formed at the interface between monomers, where the active site in one monomer faces the variable region of the adjacent monomers.

The shape of the substrate-binding pocket is highly variable between β -aminopeptidases (Merz *et al.*, 2012). The pocket determines the width of the access channel to the active site and provides the mechanism for enzyme specificity (Merz *et al.*, 2012). Although the overall structures of the β -aminopeptidases are highly conserved, it is the variation in the shape of the substrate-binding pocket of each β -aminopeptidase that contributes to the diversity of the β -aminopeptidase family. BcA5-BapA has mostly hydrophobic residues forming the substrate-binding pocket, and the positions of Trp120 and Tyr129 from adjacent monomers appear to restrict access to the active site. In this regard, it appears that BcA5-BapA is most similar to DmpA, Ps BapA and BapF, where the tryptophan residue is conserved; in 3-2W4 BapA and PH0078 there is an arginine or a serine, respectively, at this position. The substrate-binding pocket and the channel to the active site are comparatively wider and more surface-exposed in BcA5-BapA than in DmpA. DmpA has been shown to only catalyze small substrates with high efficiency owing to the presence of Trp137, which restricts active-site access for larger substrates (Faniel, Goffin *et al.*, 1999; Heck *et al.*, 2006). Mutation of the tryptophan to an alanine confirmed the gatekeeper role of the tryptophan (Merz *et al.*, 2012). Whilst BcA5-BapA has a slightly wider substrate-binding channel than DmpA, it is likely that the conserved position of the tryptophan residue plays a role in its substrate specificity.

In conclusion, this study resulted in the successful cloning, purification, crystallization and structure solution of

BcA5-BapA. This enzyme belongs to the DmpA family of Ntn hydrolases. This new structural data will be useful in the future bioengineering of the active-site residues of β -aminopeptidases for use in β -peptide synthesis, which has many potential applications in peptidomimetics and biomaterials.

Acknowledgements

We thank the Australian Synchrotron (MX1 and MX2) and the beamline scientists for beamtime (CAP8208) and for technical assistance. We thank the Monash Technology Research Platforms (Protein Production and Crystallization) for technical assistance. The authors declare that they have no conflicts of interest with the contents of this article.

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