Biochemical and Biophysical Research Communications 437 (2013) 538–543

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Structural modelling of substrate binding and inhibition in penicillin V acylase from *Pectobacterium atrosepticum*

V.S. Avinash [1], Priyabrata Panigrahi [1], C.G. Suresh, Archana V. Pundle, Sureshkumar Ramasamy *

Division of Biochemical Sciences, National Chemical Laboratory, Pune, India

ARTICLE INFO

Article history:
Received 17 June 2013
Available online 12 July 2013

Keywords:
Penicillin acylase
Bile salt hydrolase
Homology modelling
Inhibition
Pectobacterium
Docking

ABSTRACT

Penicillin V acylases (PVAs) and bile salt hydrolases (BSHs) have considerable sequence and structural similarity; however, they vary significantly in their substrate specificity. We have identified a PVA from a Gram-negative organism, *Pectobacterium atrosepticum* (*Pa*PVA) that turned out to be a remote homolog of the PVAs and BSHs reported earlier. Even though the active site residues were conserved in *Pa*PVA it showed high specificity towards penV and interestingly the penV acylase activity was inhibited by bile salts. Comparative modelling and docking studies were carried out to understand the structural differences of the binding site that confer this characteristic property. We show that *Pa*PVA exhibits significant differences in structure, which are in contrast to those of known PVAs and such enzymes from Gram-negative bacteria require further investigation.

1. Introduction

Choloylglycine hydrolases (CGH, E.C. 3.5.1.24) are a family of enzymes belonging to the Ntn hydrolase super family, which act on non-protein amide bonds. This group includes bile salt hydrolase (BSH) and penicillin V acylase (PVA) [1]. PVAs cleave the phenoxy side chain of penicillin V from the beta-lactam nucleus, and have great potential for use in the industry, in the manufacture of semi synthetic antibiotics [2]. BSHs act specifically on glyco and tauro-conjugated derivatives of cholic acid. They have been shown to occur in bacteria that normally colonise the human gut (especially *Lactobacillus* and *Bifidobacterium* genera), where they impart resistance to bile [3] and also lower blood cholesterol level [4]. BSHs also occur in certain pathogens; for instance, in *Listeria monocytogenes* [5] and *Brucella abortus* [6], they have been shown to act as a virulence factor involved in pathogenesis. On the other hand, there is no clear literature on the role of penicillin V acylase with respect to the habitat or lifestyle of the bacteria, although it has been hypothesised to act as a scavenging enzyme for degrading phenolic compounds as alternative carbon sources [7]. However, Kovacikova et al. [8] have reported that the *pva* gene from *Vibrio cholerae* is repressed by high levels of the quorum sensing-regulated virulence activator AphA which might help the bacterium to survive better in different environments.

The choloylglycine hydrolases reported so far appear to have significant differences in their activity and substrate specificity from pure BSH to pure PVA [9], with some of them able to hydrolyse both substrates. We recently identified a penicillin V acylase from *Pectobacterium atrosepticum*, a Gram negative plant pathogen that causes soft rot in potato. Although the catalytic residues were conserved, the gene sequence shared low identity with previously reported BSH and PVAs (25%), which raises the possibility that this enzyme could exhibit distinct structural and biochemical characteristics. It is also necessary to study the variation in mode of binding with different substrates and catalytic action of these enzymes from diverse classes of organisms, which might help us understand their evolution and *in vivo* function. In this study we have attempted to characterise the penicillin V acylase from *P. atrosepticum* and analyse its substrate specificity by experimental as well as comparative modelling and docking approach.

2. Materials and methods

2.1. Production and purification of penicillin V acylase from P. atrosepticum

P. atrosepticum DSM 30186 was grown in a minimal medium containing 0.4% glucose and 0.3% ammonium sulphate as carbon and nitrogen sources, respectively. A 10% inoculum in nutrient broth was used and the cells were harvested after 54 h. The enzyme was purified using a two step-process, with ammonium sulphate precipitation (50–70%) followed by anion exchange chromatography on Q-Sepharose. 25 mM NaCl in 1 mM TrisCl buffer, pH 7.8 (containing 1 mM DTT) was used for elution.

* Corresponding author.
E-mail address: s.ramasamy@ncl.res.in (S. Ramasamy).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.109

Table 1
Purification of penicillin V acylase from *P. atrosepticum* DSM 30168.

Purification step	Volume (ml)	Total activity (IU)	Total protein (mg)	Specific activity	Yield (%)	Fold-purification
Sonicate	31	60.85	556.45	0.109	100	1
50–70% Ammonium sulphate	9.5	58.13	76.93	0.756	95.53	6.94
Anion exchange chromatography (Q-Sepharose)	2.4	17.66	1.063	16.612	28.38	152.25

2.2. PVA activity assay

The activity of penicillin V acylase was determined by the method of Shewale et al. [10], measuring the amount of 6-APA formed at 40 °C, employing 2% w/v (50 mM) solution of penicillin V (potassium salt) in 0.1 M phosphate buffer pH 6. The 6-APA formed was estimated using 6% (w/v) p-dimethyl amino benzaldehyde (DAB) in methanol. One unit (IU) PVA activity is defined as the amount of enzyme that produces 1 μmol 6-APA per minute under the conditions defined.

2.3. Bile salt hydrolase assay

The hydrolysis of bile salts was estimated using 10 mM sodium glyco or taurocholate as substrate, in 0.1 M phosphate buffer pH 6 at 40 °C. The amino acid moiety released was quantified using ninhydrin reagent [9].

2.4. Biochemical characterisation of P. atrosepticum PVA

The hydrolytic activity of purified PVA towards various penicillins, cephalosporins and synthetic substrates was determined by incubating the enzyme with the different substrates at appropriate concentrations. The effect of metal ions, DTT and amino acid modifiers on PVA activity was checked to ascertain the presence of important catalytic residues at the enzyme active site.

2.5. Inhibition of PVA activity by bile salts

To study the binding of bile salts, the enzyme was incubated with increasing concentrations (10–50 mM) of glycocholic acid (GCA) and taurocholic acid (TDCA), along with the substrate (50 mM penicillin V) in the enzyme assay.

2.6. Homology modelling of 3D structure of PaPVA sequence

The amino acid sequence of *Pa*PVA gene (YP_051294.1) was obtained from protein database of NCBI (http://www.ncbi.nlm.nih.gov/protein). In order to find its closest homolog with known tertiary structure, the sequence was searched against PDB database [11] using the program blastp [12]. The best templates identified were the crystal structure of cholylglycine hydrolases from *Bacteroides thetaiotaomicron VPI* (PDB Id 3HBC) and *Bacillus sphaericus* (PDB Id 3PVA). Multiple template based homology modelling approach of "Prime 3.0" [13] was used for model building. The N-terminal 29 residue periplasmic signal peptide was removed from the *Pa*PVA sequence before building the model. First, the two templates were structurally aligned with each other. Secondary structure of *Pa*PVA sequence was predicted and was used to align the query sequence with the templates. The model building step involved copying the coordinates of backbone atoms for the aligned regions along with side chains of conserved residues. This is followed by optimisation of side chains, minimisation of non-template residues and finally building insertions and removing deletions. OPLS_2005 all-atom force field was used for energy scoring of protein and Surface Generalised Born (SGB) continuum solvation model was used for treating solvation energies. The N-terminal in 3HBC structure starts with residue Cys26. However, in the *Pa*PVA model, the residues numbering was kept such that the N-terminal Cys residue gets residue number 1. The stereo chemical quality and geometry of the final model was evaluated using model validation programs such as PROCHECK [14], Verify3D [15] and Prosa [16].

2.7. Prediction of penV, GCA binding modes

The 3D conformation of glycocholic acid (GCA) and penicillin V (penV) used in the docking study was obtained from PubChem compound database [17] with their CID 10140 and 6869, respectively. Partial atomic charges of each ligand atom were determined from OPLS_2005 all-atom force field using LigPrep [18]. Grid based ligand docking program Glide [19] was used for docking these ligands in the binding site of receptor. The binding site was defined as a grid box of dimension 26 × 26 × 26 Å, centered on Cys1 residue. Receptor grid generation was followed by ligand docking where the ligands were docked flexibly using Glide's extra precision. Free energy of binding is roughly estimated by using an empirical scoring function called GlideScore, which includes electrostatic, van der Waals interaction and other terms for rewarding or penalising interactions that are known to influence ligand binding.

3. Results and discussion

The enzymes PVA and BSH have immense potential as commercial enzymes, in the pharmaceutical [2] or food industry [20]. In addition, these enzymes are also possibly linked to the regulation of pathogenicity in bacteria. Structures have been solved for PVA from *B. sphaericus* (*Bsp*PVA, PDB ID 3PVA) [21] and *B. subtilis* (*Bsu*PVA, 2OQC) [22], and BSH from *Clostridium perfringens* (*Cp*BSH, 2RLC) [23] and *Bifidobacterium longum* (*Bl*BSH, 2HF0) [9]. Even though these enzymes have appreciable sequence homology, they exhibit significant variation in substrate specificity. *Bsu*PVA and *Bl*BSH are exclusively specific for penicillin V and glyco-conjugated bile salts, respectively. On the other hand, *Bsp*PVA and *Cp*BSH show some cross-activity [9].

Based on this logic, we expected that the penicillin V acylase from *P. atrosepticum*, a Gram-negative bacterium, might exhibit distinct biochemical and structural characteristics different than the sources reported earlier. Hence we proceeded to characterise the enzyme and study the substrate binding to explore the unique nature of this enzyme. The enzyme was purified using ion exchange chromatography and observed to be active at pH 5.5 and 40 °C. A 152-fold increase in specific activity of enzyme to 16.6 μM/mg/min of protein and 28% recovery of the total activity was achieved (Table 1). The value for specific activity is less than that reported for *B. sphaericus* PVA [24].

3.1. Biochemical characteristics of PaPVA

The activity of *Pa*PVA was not influenced by most metal ions or chelating agents (data not shown). However, the enzyme activity was inhibited largely in presence of Ag^{+}, Hg^{2+}, and Cd^{2+} ions. These cations have strong affinities for –SH groups, indicating that the active site of PVA may contain cysteine. There was also a marked enhancement in activity after treatment with DTT (126% of original activity at 10 mM), which is in agreement with previous findings for penicillin V acylases [25]. The enzyme was inhibited to a large

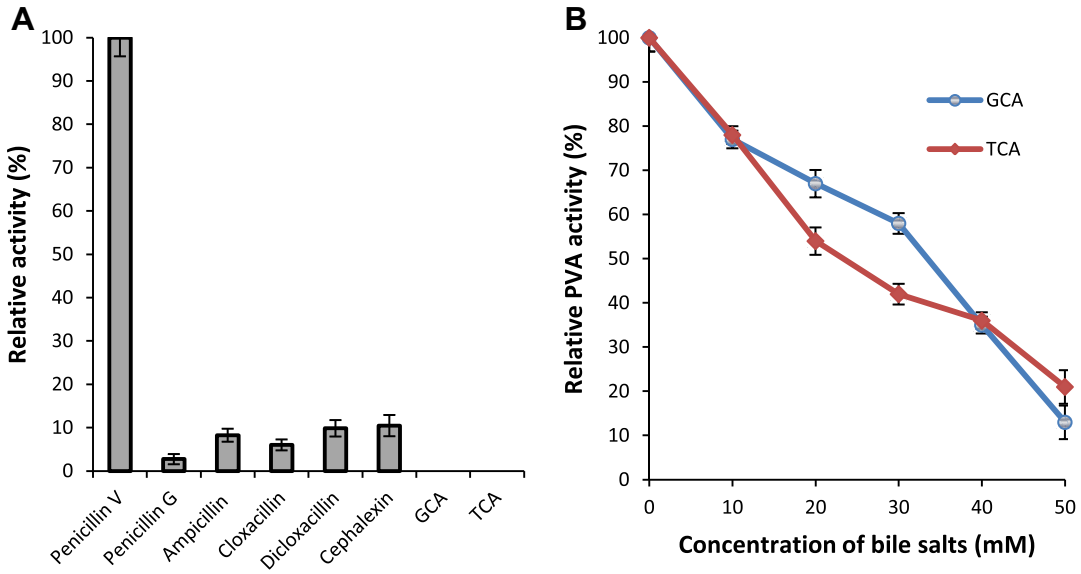

Fig. 1. (A) Substrate specificity of *Pa*PVA for beta-lactam compounds and bile salts. Activity towards GCA and TCA was measured using BSH assay. (B) Inhibition of *Pa*PVA activity in the presence of bile salts.

extent by modification with pHMB (0.1 mM), NBS (0.1 mM) and phenyl glyoxal (10 mM); suggesting the possibility of cysteine, tryptophan or arginine residues, respectively, in the active site (Table S1). This indicates that the active site residues are probably conserved in *Pa*PVA like previously reported enzymes of this family [21].

3.2. Substrate specificity of PaPVA

Penicillin V was found to be the best substrate for *Pa*PVA since the enzyme showed highest specificity towards penV. All other beta-lactam substrates, including penicillin G, ampicillin, cephalexin, cloxacillin and dicloxacillin were hydrolysed by PVA at a rate less than 10% of that of penV. Pundle and Sivaraman [24] have also reported high specificity towards penV in the case of *Bsp*PVA. Since PVA and BSH are closely related, we examined if the *Pa*PVA enzyme could hydrolyse bile salts as well. However, the enzyme failed to show any activity with glyco- or tauro-conjugated bile salts (Fig. 1A).

To understand the reason for this variation in substrate specificity towards penV and bile salts, molecular modelling of the *Pa*PVA sequence was employed, followed by computational docking of these substrates.

3.3. Homology modelling of PaPVA

Blast homology search of *Pa*PVA sequence against PDB database identified 3HBC, a cholylglycine hydrolase from *B. thetaiotaomicron VPI*, as best template having 44% identity with query sequence followed by 3PVA (25% identity), a cholylglycine hydrolase from *B. sphaericus* [21]. In the template 3HBC, coordinates of few residues near the substrate binding site (residues 48–49, 157–162 and 271–273) were missing. When the query sequence was aligned with template 3HBC, an insertion of 5 residues (GRKGM) was found in the query sequence between residues 87 and 88 of template. Hence any region of the query sequence which could not be modelled by using 3HBC was modelled using the second template 3PVA.

Multiple template based modelling was reliable because both the templates and target belong to same enzyme family and share αββα Ntn hydrolase structural fold (Fig. 2). Majority of the secondary structure elements along with the key catalytic residues (Cys1, Arg17, Asp20, Asn175 and Arg225; Numbering as per 3PVA) are well conserved [26]. However, a significant difference was observed between the PVA enzyme from *P. atrosepticum* (*Pa*PVA) and *B. sphaericus* (*Bsp*PVA). Near the C-terminal, a 20 residue secondary structural motif (residues 193–213) which is responsible for the oligomeric association of *Bsp*PVA is completely missing in *Pa*PVA (blue[2] region in Fig. 2). Another significant difference observed was the physical nature of binding site pocket. The binding site pocket of *Bsp*PVA is large and more hydrophobic compared to that of *Pa*PVA (Fig. S2). Similarly there is a difference in the electrostatic potential, observed between the active sites of *Pa*PVA and *Bsp*PVA (Fig. S2). The hydrophobic binding site surface area in *Bsp*PVA, as calculated by tool SiteMap [27], is 261.78 Å^2 whereas in case of *Pa*PVA, it is 145.09 Å^2. The approximate binding site volume in *Bsp*PVA is 399.59 Å^3, which is slightly larger than *Pa*PVA where the binding site volume is 329.6 Å^3. The model built was of good quality, as validated by the model validation program such as Recheck, Verify3D and Prosa. More than 93% of the residues lie in the core and allowed region of Ramachandran plot as estimated by Procheck (83.9% in core, 9.7% allowed, 3.7% generously allowed and 2.7% disallowed region). All residues had a Verify3D quality score greater than zero, suggesting the absence of conformational error. The overall *Z*-score using Prosa (−6.97) was within the range of scores usually observed for native proteins of similar size, further validating the quality of modelled structures. The root mean square deviation (RMSD) of their backbone atoms was 1.39 Å.

3.4. Binding modes of substrate penV and GCA

Fig. 3 depicts the binding modes of substrate penV and GCA in the binding site of *Pa*PVA. The estimated values for free energy of binding suggested a favourable binding for both the molecules but penV exhibited higher binding affinity as compared to GCA (penV: −4.4, GCA:−3.4 kcal/mol). Both molecules interact with the key catalytic residues such as C1, D22, W23, W87, N183 and

[2] For interpretation of color in Figs. 2 and 4, the reader is referred to the web version of this article.

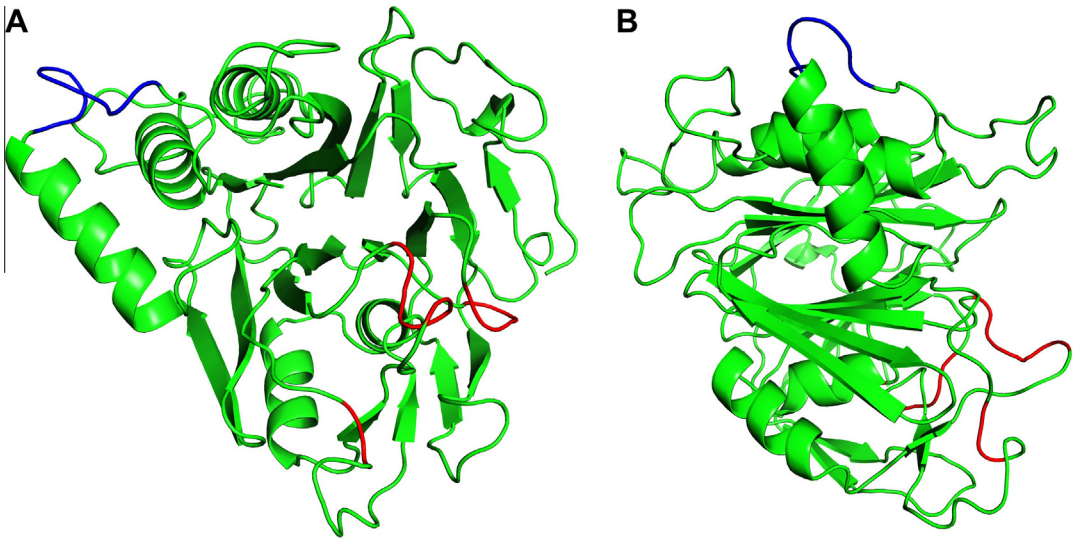

Fig. 2. (A) Three-dimensional model of *Pa*PVA represented by cartoon representation. The region shown in red are modelled using *Bsp*PVA (3PVA) as template. The region shown in blue corresponds to the structural motif responsible for the oligomeric association of *Pa*PVA. (B) Same as (A) viewed by 90° clockwise rotation about *y*-axis, showing clearly the αββα structural fold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

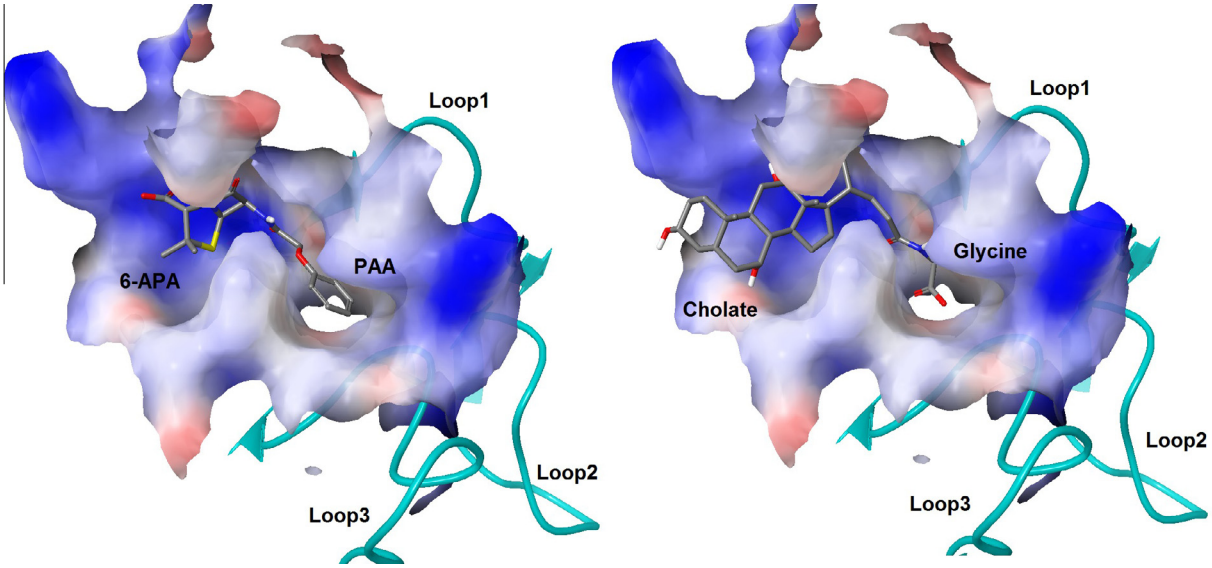

Fig. 3. The mode of binding of penV (left panel) and GCA (right panel) in the active site of *Pa*PVA. The substrate binding site loops of the enzyme, along with the leaving and tetrahedral intermediate forming groups of each ligand are labelled. Binding site is shown in electrostatic surface.

R215. In the case of penV, the residues C1 and R215 are involved in hydrogen bonding interaction with its carbonyl oxygen of the amide bond and the carboxylate group of β-lactam ring. Similarly, the aromatic residues W23 and W87 interact with the phenyl ring of penV. GCA also interacts with residues C1 and W87 (main chain nitrogen atom acts as donor) via hydrogen bonding interaction. These observations matched well with the results of chemical modification described earlier, suggesting that these residues are involved in interaction with the substrate. Further, it could be inferred that GCA can bind to the enzyme at the site where penV binds, although the enzyme did not show any activity on GCA. We proceeded to confirm this experimentally by studying the inhibition of PVA activity by bile salts.

3.5. Binding of bile salts reduces PVA activity

The addition of bile salts along with the substrate (penicillin V) in the enzyme assay led to the inhibition of PVA activity as anticipated from the docking studies. Both glyco- and tauro-conjugated bile salts showed similar patterns of inhibition of the enzyme activity, leading to almost 85% reduction at equimolar concentrations of the substrate penVand GCA (Fig. 1B).Based on our computational and experimental data, we could conclude that the enzyme from *P. atrosepticum* is active exclusively towards penV, even though bile salts also bind at the same site as penV to inhibit the catalytic activity. Rathinasamy et al. [25] have reported a slight inhibition of *Bsu*PVA activity in the presence of 1 mM bile salts.

A probable reason for such inhibition could be the inverse mode of binding of substrate penV and the inhibitor GCA (Fig. 3). In penV, after the cleavage of its amide bond, the putative group which leaves first, henceforth called the Leaving Group (LG) is 6-amino penicillanic acid (6-APA) where as in case of GCA it is the glycine moiety which leaves first. After the LG is removed, the remaining group which forms a tetrahedral intermediate with the enzyme, henceforth called Tetrahedral intermediate forming group (TG), are phenyl acetic acid (PAA) and cholate, respectively in penV

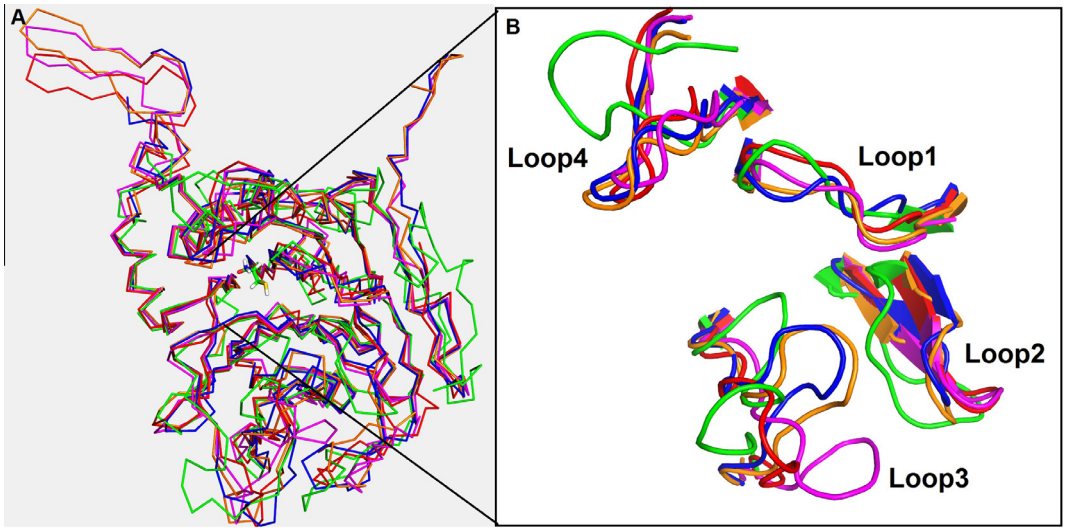

Fig. 4. (A) The superposition of CA-trace of *Pa*PVA enzyme with *Bl*BSH (red), *Cp*BSH (magenta), *Bsp*PVA (orange) and *Bsu*PVA (blue). The N-terminal catalytic residue Cys1 is shown in stick representation. (B) Zoom view of the catalytic site showing the differences in the orientation of the four substrate binding site loops. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and GCA. We observed that the location of LG and TG between substrate penV and inhibitor GCA were interchanged and hence the direction of amide bond (CO–NH direction) got reversed. Again the binding mode of GCA in *Pa*PVA is significantly different from that of *Cp*BSH (2RLC, Fig. S3). It is likely that this mode of GCA binding is not optimal for GCA cleavage, hence the absence of catalyticactivity although it binds at the same site as penV.

The structural superposition of the binding site of *Pa*PVA with other reported 3D structures of *Bl*BSH, *Cp*BSH, *Bsp*PVA and *Bsu*PVA is represented in Fig. 4. A significant difference was observed in the length and orientation of the four loops near the substrate binding site between *Pa*PVA and the other enzymes mentioned above. In *Pa*PVA these loops correspond to the following regions: Loop1: 22–35, Loop2: 61–74, Loop3: 135–150 and Loop4: 263–275. Loop1 and Loop4 are of same length in all the above enzymes. However, in *Pa*PVA, Loop2 is about 3 residues larger than the corresponding loop in *Bl*BSH, *Cp*BSH and *Bsp*PVA enzymes and about 4 residues larger compared to *Bsu*PVA Loop2. This insertion causes the loop to fold further inwards towards the active site, decreasing its size. Except *Bsp*PVA, all other enzymes have one or two residue shorter Loop3 compared to *Pa*PVA. It has been suggested earlier that the variation in specificity towards GCA and penV between BSH and PVA enzymes is due to these loops [9,28]. In PVA enzymes, these loops are folded inward forming a closed and reduced size binding site pocket whereas the pocket is comparatively large and open in the case of BSH. In *Pa*PVA, due to insertion of 3–4 residues in Loop2, about 1–2 residues in Loop3 and their orientation, the binding site pocket was further reduced in its size, preventing GCA to bind in a mode similar to the mode seen in *Cp*BSH (2RLC).Structural superposition of *Pa*PVA with 2RLC showed heavy steric clashes between the bound product cholate of CpBSH and the residues of *Pa*PVA Loop2 and Loop3 (Fig. S3).

In conclusion, we have characterised a penicillin V acylase from a Gram-negative bacterium (*P. atrosepticum*), which shows activity towards penV and related compounds, but fails to hydrolyse bile salts. Computational docking showed inverse or reverse mode of binding of the substrate to the enzyme, this probably accounts for the inhibition of PVA activity in the presence of bile salts. The orientation of the loops in *Pa*PVA accounts for a reduction in the size of the binding pocket which makes it highly specific for penV. Such information on the structural differences in binding can be particularly valuable to design strategies for protein engineering to improve the enzyme characteristics. In addition, based on sequence and structural homology, we observed that *Pa*PVA was distinct from already reported PVA and BSH enzymes, hinting at the possibility that PVAs from Gram-negative organisms might belong to a different class [28]. It would also be interesting to study the docking of plant phenolic compounds in the active site of *Pa*PVA to ascertain whether such compounds can act as better substrates than penV.

Acknowledgments

V.S.A. and P.P. thank Council of Scientific and Industrial Research (CSIR), India for the award of Senior Research Fellowship. R.S.K. thanks the Department of Science and Technology (DST), Government of India for Ramanujan Fellowship. This work was supported by CSIR network project- HUM.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.109.

References

[1] H. Christiaens, R.J. Leer, P.H. Pouwels, W. Verstraete, Cloning and expression of a conjugated bile-acid hydrolase gene from *Lactobacillus plantarum* by using a direct plate assay, Appl. Environ. Microbiol. 58 (1992) 3792–3798.
[2] J.G. Shewale, V.K. Sudhakaran, Penicillin V acylase: its potential in the production of 6-aminopenicillanic acid, Enzyme Microb. Technol. 20 (1997) 402–410.
[3] I. Desmet, L. Vanhoorde, M.V. Woestyne, H. Christiaens, W. Verstraete, Significance of bile-salt hydrolytic activities of Lactobacilli, J. Appl. Bacteriol. 79 (1995) 292–301.
[4] F.A.M. Klaver, R. Vandermeer, The assumed assimilation of cholesterol by Lactobacilli and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity, Appl. Environ. Microbiol. 59 (1993) 1120–1124.
[5] O. Dussurget, D. Cabanes, P. Dehoux, M. Lecuit, C. Buchrieser, P. Glaser, P. Cossart, *Listeria monocytogenes* bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis, Mol. Microbiol. 45 (2002) 1095–1106.
[6] M.V. Delpino, M.I. Marchesini, S.M. Estein, D.J. Comerci, J. Cassataro, C.A. Fossati, P.C. Baldi, A bile salt hydrolase of *Brucella abortus* contributes to the establishment of a successful infection through the oral route in mice, Infect. Immun. 75 (2007) 299–305.
[7] F. Valle, P. Balbas, E. Merino, F. Bolivar, The role of penicillin amidases in nature and in industry, Trends Biochem. Sci. 16 (1991) 36–40.
[8] G. Kovacikova, W. Lin, K. Skorupski, The virulence activator AphA links quorum sensing to pathogenesis and physiology in *Vibrio cholerae* by repressing the expression of a penicillin amidase gene on the small chromosome, J. Bacteriol. 185 (2003) 4825–4836.

[9] R.S. Kumar, J.A. Brannigan, A.A. Prabhune, A.V. Pundle, G.G. Dodson, E.J. Dodson, C.G. Suresh, Structural and functional analysis of a conjugated bile salt hydrolase from *Bifidobacterium longum* reveals an evolutionary relationship with penicillin V acylase, J. Biol. Chem. 281 (2006) 32516–32525.
[10] G.J. Shewale, K.K. Kumar, G.R. Ambekar, Evaluation of determination of 6-aminopenicillanic acid by p-dimethyl aminobenzaldehyde, Biotechnol. Tech. 1 (1987) 69–72.
[11] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The protein data bank, Nucleic Acids Res. 28 (2000) 235–242.
[12] S.F. Altschul, T.L. Madden, A.A. Schaffer, J.H. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–3402.
[13] Prime, version 3.0, Schrodinger, LLC, New York, NY, 2011.
[14] R.A. Laskowski, M.W. Macarthur, D.S. Moss, J.M. Thornton, PROCHECK – a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283–291.
[15] D. Eisenberg, R. Luthy, J.U. Bowie, VERIFY3D: assessment of protein models with three-dimensional profiles, Methods Enzymol. 277 (1997) 396–404.
[16] M. Wiederstein, M.J. Sippl, ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins, Nucleic Acids Res. 35 (2007) W407–W410.
[17] E. Bolton, Y. Wang, P.A. Thiessen, S.H. Bryant, PubChem: Integrated platform of mall molecules and biological activities, in: Annual Reports in Computational Chemistry, Elsevier, Washington DC, 2008, pp. 217–241.
[18] LigPrep, version 2.5, Schrodinger, LLC, New York, NY, 2012.
[19] Glide, version 5.8, Schrodinger, LLC, New York, NY, 2012.
[20] M. Begley, C. Hill, C.G.M. Gahan, Bile salt hydrolase activity in probiotics, Appl. Environ. Microbiol. 72 (2006) 1729–1738.
[21] C.G. Suresh, A.V. Pundle, H. SivaRaman, K.N. Rao, J.A. Brannigan, C.E. McVey, C.S. Verma, Z. Dauter, E.J. Dodson, G.G. Dodson, Penicillin V acylase crystal structure reveals new Ntn-hydrolase family members, Nat. Struct. Mol. Biol. 6 (1999) 414–416.
[22] P. Rathinaswamy, A.V. Pundle, A.A. Prabhune, H. SivaRaman, J.A. Brannigan, G.G. Dodson, C.G. Suresh, Cloning, purification, crystallization and preliminary structural studies of penicillin V acylase from *Bacillus subtilis*, Acta Crystallogr. Sect. F: Struct. Biol. Cryst. Commun. 61 (2005) 680–683.
[23] M. Rossocha, R. Schultz-Heienbrok, H. von Moeller, J.P. Coleman, W. Saenger, Conjugated bile acid hydrolase is a tetrameric N-terminal thiol hydrolase with specific recognition of its cholyl but not of its tauryl product, Biochemistry 44 (2005) 5739–5748.
[24] A. Pundle, H. SivaRaman, *Bacillus sphaericus* penicillin V acylase: purification, substrate specificity, and active-site characterization, Curr. Microbiol. 34 (1997) 144–148.
[25] P. Rathinaswamy, S.M. Gaikwad, C.G. Suresh, A.A. Prabhune, J.A. Brannigan, G.G. Dodson, A.V. Pundle, Purification and characterization of Yxel, a penicillin acylase from *Bacillus subtilis*, Int. J. Biol. Macromol. 50 (2012) 25–30.
[26] A. Lodola, D. Branduardi, M. De Vivo, L. Capoferri, M. Mor, D. Piomelli, A. Cavalli, A catalytic mechanism for cysteine N-terminal nucleophile hydrolases, as revealed by free energy simulations, PLoS One 7 (2012) e32397, http://dx.doi.org/10.1371/journal.pone.0032397.
[27] SiteMap, version 2.5, Schrodinger, LLC, New York, NY, 2011.
[28] J.M. Lambert, R.S. Bongers, W.M. de Vos, M. Kleerebezem, Functional analysis of four bile salt hydrolase and penicillin acylase family members in *Lactobacillus plantarum* WCFS1, Appl. Environ. Microbiol. 74 (2008) 4719–4726.