NE3A/2012G658

Crystal Structures of the Pen β-Lactamases of Burkholderia cepacia (Bc) and Burkholderia pseudomallei (Bp)

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1 Introduction

Resistance to penicillins and cephalosporins in Bc and Bpis a serious concern. Bc causes infections in patients with cysticfibrosis and Bp is responsible for fatal pneumonia (meliodosis). PenA and PenI, unique class A β-lactamases from Bc and Bp, respectively, are homologous by amino acid sequences with 60.9% identity. In addition, they are more similar to P. vulgaris K1 (48.1/49.2),CTX-M-9 (51.8/51.5) and Toho-1 (53.3/47.8) than TEM-1 (39.0/38.7) and SHV-1 (42.2/40.4) β-lactamases (% identity to PenA/PenI). P. vulgaris K1, CTX-M and Toho-1 β-lactamases are extended-spectrum β-lactamases with ability to hydrolyze extended-spectrum cephalosporins and monobactams, while PenA and PenI are not. Our goal was to decipher the crystal structures of the PenA and PenI β-lactamases in order to serve as a starting point for intelligent inhibitor design.

2 Experiment

PenA and PenI β-lactamases were expressed in Escherichia coli Origami2 (DE3), purified, and crystallized by vapor diffusion method using a 250 μL reservoir with 4 μL hanging drop (2 μL reservoir solution + 2 μL protein solution). For PenA, the well solution contained 25% polyethylene glycol of 8 kilodaltons (PEG8K), 0.2 M sodium chloride, 0.1 M sodium phosphate/citric acid at pH 4.2 and 15 mg/ml of PenA in 2 mM 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES), pH 7.5. Two successful conditions were obtained for PenI, one at pH 7.5 and the other at pH 9.5. One well solution contained 25% PEG8K, 0.1M ammonium sulphate, 0.1M HEPES, pH 7.5, and 10 mg/ml protein in 2 mM HEPES, pH7.5, while the secondcontained 25% PEG8K, 0.1 M N-cyclohexyl-2-aminoethanesulfonic acid pH 9.5 (reservoir), and 10 mg/ml protein in 2 mM HEPES, pH 7.5. Crystals appeared in 1 - 2 weeks reaching sizes of 0.4 - 0.8 mm. The crystals were cryoprotected by dipping them into a reservoir solution containing 20% glycerol, flush-cooled, and kept at 100K with a nitrogen gas stream.

The 0.5º oscillation images were collected on a Q270 CCD detector with synchrotron radiation (λ = 1.00Å at beam-line NE3A of the Advanced Ring of the Photon Factory, Tsukuba, Japan). The HKL2000 program was used to index and scale X-ray intensities. For the PenI structure, the β was close to 90º (89.996º as the HKL2000 output) in the *C*2 space group. Other bravais-lattice candidates, which were suggested by HKL2000 were also examined. Only in the case of the C Centered Monoclinic, was a reasonable Rmerge value (0.04 vs > 0.4) obtained.

3 Results and Discussion

The PenA crystal structure was obtained at a 1.2 Å resolution at pH 4.2 and the PenI structures were obtained at 1.05 Å resolution at pH 7.5 and at 1.18 Å resolution at pH 9.5. The PenA β-lactamase crystal was in the 6 space group *C*2 with three molecules per asymmetric unit (Table 1). The PenI β-lactamases at pH 7.5 and pH 9.5 were both crystallized in space group *P*21 with one molecule in the asymmetric unit (Table 1).

Table 1. X-ray data collection and crystallization refinement statistics

	PenI at pH9.5	PenI at pH7.5	PenA at pH4.2
Data Collection			
Space group	$P2_1$	$P2_1$	*C*2
Cell constant	41.4, 52.8, 50.5,	41.4, 52.7, 50.5,	121.0, 69.9, 84.4,
a,b,c, (Å)	90.0, 92.6, 90.0	90.0, 92.5, 90.0	90.0, 90.0, 90.0
Wavelength (Å)	1.000	1.000	1.000
Resolution (Å)	50-1.18(1.22-1.18)	50-1.05(1.07-1.05)	50-1.20(1.22-1.20)
Observations	274380(21669)	375483(8696)	845028(36428)
Unique reflections	68595(6191)	98393(3781)	208965(10119)
Rmerge	0.088(0.286)	0.092(0.350)	0.039(0.450)
Completeness (%)	96.4(87.5)	97.8(75.7)	95.5(93.1)
Av $I/\sigma(I)$	14.8(4.3)	13.6(2.6)	31.6(2.7)
Data Refinement			
Resolution range	15-1.18	15-1.05	15-1.2
No. of reflections used [$F > 0\sigma(F)$]	66491	95315	202528
R_{work}/R_{free} [a]	0.1138/0.1528	0.1345/0.1602	0.1322/0.1749
R_{total}	0.1150	0.1354	0.1334
Residue in Ramachandran zone			
Favored/allowed	260(98.5%)/4(1.5%)	262(99.2%)/2(0.8%)	759(98.8%)/9(1.2%)
Disallowed	0	0	0
RMSD values from ideality			
Bond lengths (Å)	0.013	0.014	0.013
Bond angles (Å)	0.031	0.029	0.031
Zero chiral volumes ($Å^3$)	0.080	0.081	0.074
Nonzero chiral volumes ($Å^3$)	0.088	0.096	0.080
Mean *B*-factor (no. of atoms)			
Protein	9.35(2130)	12.57(2104)	16.39(6039)
Solvent	21.83(249)	26.27(171)	31.16(929)
Others	14.06(19)	35.38(23)	none
Total	10.68(2398)	13.82(2298)	18.36(6968)
No. of hydrogen atoms	2137	2088	5949

[a] R_{free} values were calculated from 3% of reflections, respectively.

Despite differences in amino-acid sequence, PenA and PenI (pH 7.5) have very similar overall three-dimensional structures (i.e., RMSD = 0.59 Å for Cα atoms between positions 32-289). To identify differences that may contribute to the contrasting-kinetic properties of PenA and PenI, the Ser70Xaa71Xaa72Lys73 motif, Ser130Asp131Asn132 loop, Lys234Thr235Gly236 motif, B3 β-strand, 102-110 loop, and Ω loop were compared; RMSD values comparing PenA to PenI (pH .5) are 0.41 Å, 0.53 Å, 0.23 Å, 0.49 Å, and 0.27 Å for these motifs, respectively (Fig 1).

The Ser70Xaa71Xaa72Lys73 motif contains the nucleophilic Ser-70 as well as Lys-73, which can serve as a general base to initiate β-lactam acylation and is also involved in the proton shuttle during β-lactam hydrolysis. Fig 2A shows that two conformations of Lys-73 are found

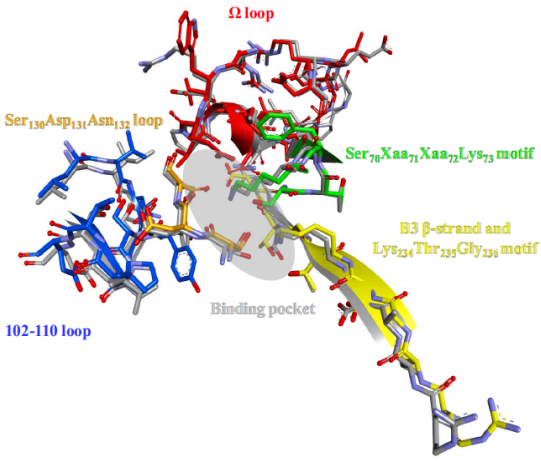

Fig. 1: Overlay of the PenA (colored) and PenI (gray) active sites highlighting the major motifs: Ser70Xaa71Xaa72Lys73 motif (green), Ser130Asp131Asn132 loop (orange), Lys234Thr235Gly236 motif (yellow), B3 β-strand (yellow), 102-110 loop (blue), and Ω loop (red) and the approximate-binding pocket (gray circle) for β-lactams and β-lactamase inhibitors.

in the structure of PenI obtained at pH 9.5. An overall decreased-hydrolytic profile is observed in PenI compared to PenA and the positioning of Lys-73 may be an important-contributing factor. In conformation 1, which is identical to the conformation observed in PenA (cyan), the Nζ of Lys-73 can form hydrogen bonds with the Oδ1 of Asn-132 (distance 2.6 Å) and the Oγ of Ser-70 (distance 2.5 Å), while in conformation 2, the Nζ of Lys-73 is within hydrogen-bonding distance of the Oε1 of Glu-166 (distance 2.6 Å) and the Oγ of Ser-70 (distance 2.9 Å). Not only are Lys-73 and Ser-70 important for β-lactam hydrolysis, but Glu-166 can also serve as a general base important for acylation and deacylation of β-lactams. As a consequence, both the interactions observed between Lys-73, Glu-166, and Ser-70 in conformation 2, as well as the ability of Lys-73 to have two conformations, may decrease substrate hydrolysis due to inhibiting the proper function of these residues (e.g, decreased ability of Lys-73 to abstract a proton from Ser-70, altered proton shuttling, and changes in the pKa of Glu-166).

The Ω loop comprises residues 164 to 179 and contains two critical-active-site residues, Glu-166 and Asn-170; both residues anchor the deacylation-water (DW) molecule required for β-lactam hydrolysis.
Hydrogen-bonding interactions between DW molecules and the three structures are different. In the PenA structure the DW molecule forms hydrogen bonds with the Oε2 of Glu-166, the Nδ2 of Asn-170, the N of Ser-70, and Oγ of Ser-70. Compared to PenA (Fig 2A), in PenI at pH 7.5 and pH 9.5, the side chains of Asn-170 and Glu-166 are rotated 180º, thus the hydrogen-bonding interactions with the DW are different (Fig 2A). Specifically, in the pH 7.5 structure, the DW is not anchored appropriately to Asn-170; this suggests that the DW may be impaired in promoting deacylation of the β-lactam. In addition, the rotation of Glu-166 changes the hydrogen-bonding partner from the Oε2 of Glu-166 to the Oε1 of Glu-166 with the DW. In the PenI structure at pH 9.5, the DW possesses additional hydrogen bonds to the Nδ2 and Oδ1 of Asn-170, which may decrease the rate of deacylation. In addition to the alterations in hydrogen-bonding pattern of the DW in the three structures, the DW molecule is at a lower occupancy in PenI about 25% (21.7% at pH 7.5 and 27.4% at pH 9.5) compared to PenA, which is at 100% (Fig 2B and 2C). The lower DW occupancy may further explain why PenI demonstrates lower-catalytic activity compared to PenA.

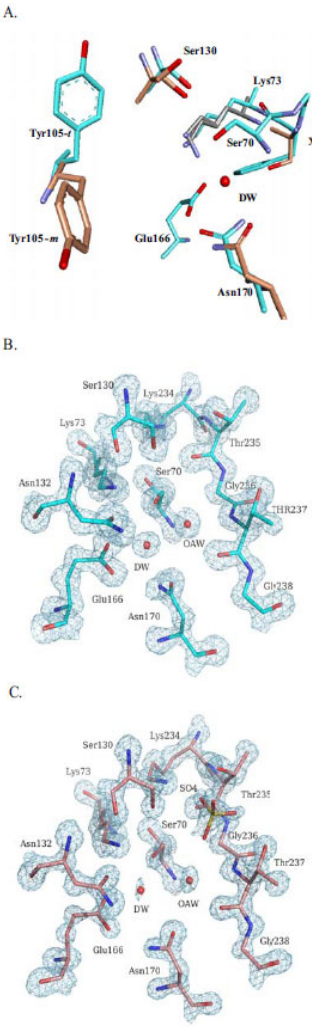

Fig. 2: Crystal structures of PenA and PenI. A. Overlay of active site of PenA (cyan), PenI at pH 7.5 (orange), and PenI at pH 9.5 (gray) highlighting the alternate conformations of Lys-73, Ser-130, Tyr-105, and Asn-170. B. Electron density of PenA active site showing the occupancy of the DW moleculeis at 100%. Also represented is the water molecule in the oxyanion hole (OAW). C. Electron density ofPenI pH7.5 active site showing the occupancy of the DW molecule is at 21.7%. Also represented is the water molecule in the oxyanion hole (OAW).

References

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