BL-5A/2011G237

Structures of Staphylococcus aureus peptide deformylase in complex with two classes of new inhibitors

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1 Introduction

Peptide deformylase (PDF) catalyzes the removal of the formyl group from the N-terminal methionine residue in newly synthesized polypeptides, which is an essential process in bacteria. PDF, a metalloenzyme that is highly conserved in bacteria, has been proposed as one attractive such target Therefore, we have developed four new inhibitors of PDF that belong to two different classes, hydroxamate/pseudopeptide compounds [PMT387 (7a) and PMT497] and reverse-hydroxamate/nonpeptide compounds [PMT1039 (15e) and PMT1067]. These compounds inhibited the growth of several pathogens involved in respiratory-tract infections, such as *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae*, and leading nosocomial pathogens such as *Staphylococcus aureus* and *Klebsiella pneumonia* with a minimum inhibitory concentration (MIC) in the range 0.1–0.8 mg/ml. pneumoniae. Of these inhibitors, PMT1039 (15e) and PMT1067, with nonpeptide scaffolds, were highly effective in inhibiting the *S. aureus* and *K. pneumoniae* PDFs. When we compared and analyzed the four new inhibitor bound PDF structures, distinct structural changes that were dependent on the inhibitor class were observed. The structural changes appear to be driven by the binding of the inhibitor and are likely to be involved in inhibition, particularly of *S. aureus*.

2 Experiment

Purified *S. aureus* PDF and the inhibitors were mixed in a molar ratio of 1:6.6 at 24°C for 1 hour and crystallized by hanging drop vapor diffusion at 24°C, by mixing equal volumes (2 ml each) of protein solution (30 mg/ml concentration in 20 mM Tris–HCl buffer pH 7.5 containing 120 mM NaCl) and reservoir solution consisting of 23%(w/v) PEG 4000, 50 mM Tris–HCl pH 8.5, 15%(v/v) glycerol, 100 mM $MgCl_2$, 20 mM $CaCl_2$. A model of *S. aureus* PDF (Yoon et al., 2004) was used as a search model. The models were built manually using the program Coot and refined with the program REFMAC.

3 Results and Discussion

The crystals belonged to the orthorhombic space group $C222_1$. One monomer was present in each asymmetric unit of the crystal. The inhibitor compounds [PMT387 (7a), PMT497, PMT1039 (15e), PMT1067 and actinonin] were synthesized and MIC tests were performed (Table 1). There were measurable structural differences between the four inhibitor-bound *S. aureus* PDF structures and an inhibitor-free structure. Despite the high rigidity of the *S. aureus* structure, two different classes of PDF inhibitors induced local structural changes. We observed that these structures displayed distinct structural features depending on the inhibitor class. The distances between the hydroxamate carbonyl O atom and the Zn^{2+} ion and between the reverse-hydroxamate hydroxyl O atom and the Zn^{2+} ion in the hydroxamate or reverse-hydroxamate moiety appeared to be correlated with selective and strong inhibition activity against *S. aureus*.

Inhibitors	MIC (μg/ml)					
	S. aureus (ATCC 6538p)	*K. pneumoniae* (ATCC 10031)	*E. coli* (ATCC 25922)	*S. pneumoniae* (ATCC 6305)	*M. catarrhalis* (ATCC 43617)	*H. influenzae* (ATCC 49247)
	Gram (+)	Gram (-)	Gram (-)	Gram (+)	Gram (-)	Gram (-)
PMT387	50	0.4–0.8	25	0.2[a]	0.1[a]	0.2[a]
PMT497	50	6.3	25	0.8	0.1	0.1
PMT1039	0.2[a]	0.1	12.5	0.1–0.2[a,b]	0.1[a]	0.1[a]
PMT1067	0.2	0.4	12.5	0.1[a]	0.4	0.1
Actinonin	6.3–12.5	0.8–3.2	25–50	3.2–6.3	0.1–0.2	1.6–3.2
Ampicillin	0.2	25–100	1.6	0.1[a]	0.1–0.4	3.2
LBM-415	2[c]	32[c]	32[c]	1[c]	0.5[c]	4–8[c]

Table 1: Results of enzymatic assays and MICs.

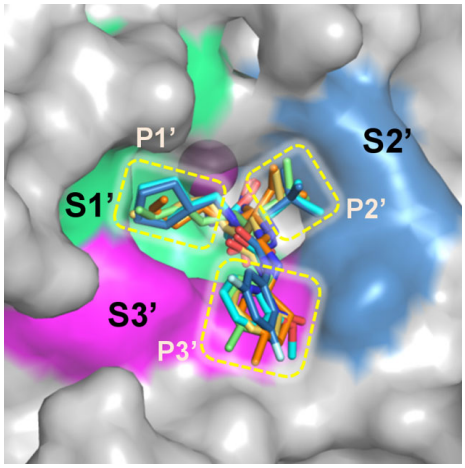

Fig. 1: Surface diagram of *S. aureus* PDF with the active-site pocket depicted. The molecular surface is coloured green (S1'), blue (S2') and magenta (S3'). The P1', P2' and P3' residues of the four inhibitors corresponding to the S1', S2' and S3' sites are indicated by yellow dashes. The Zn^{2+} ion is shown as a sphere.

Reference

[1] S.J. Lee *et al*., Acta Cryst, D68, 784-793 (2012).