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Structural and Binding Studies of Peptidyl-tRNA Hydrolase from Pseudomonas aeruginosa Provide a Platform for the Structure Based Inhibitor Design against Peptidyl-tRNA Hydrolase

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Abstract : Peptidyl-tRNA hydrolase (Pth) Pth is an essential bacterial enzyme that catalyzes the release of free tRNA and peptide moeities from peptidyl tRNAs during stalling of protein synthesis. In order to design inhibitors of Pth from Pseudomonas aeruginosa (PaPth), we have determined the structures of PaPth in its native state and in the bound states with two compounds, amino acylate-tRNA analogue (AAtA) and 5-azacytidine (AZAC). The peptidyl-tRNA hydrolase gene from Pseudomonas aeruginosa was amplified by Phusion High-Fidelity DNA Polymerase using forward and reverse primers, respectively. The E. coliBL21 (λDE3) strain was used for expression of the recombinant peptidyl-tRNA hydrolase from Pseudomonas aeruginosa. The protein was purified using a Ni-NTA superflow column. The crystallization experiments were carried out using hanging drop vapour diffusion method. The crystals diffracted to 1.50 Å resolution. The data were processed using HKL-2000. The polypeptide chain of PaPth consists of 194 amino acid residues from Met1 to Ala194. The centrally located β-structure is surrounded by α-helices from all sides except the side that has entrance to the substrate binding site. The structures of the complexes of PaPth with AAtA and AZAC showed the ligands bound to PaPth in the substrate binding cleft and interacted with protein atoms extensively. The residues that formed intermolecular hydrogen bonds with the atoms of AAtA included Asn12, His22, Asn70, Gly113, Asn116, Ser148, and Glu161 of the symmetry related molecule. The amino acids that were involved in hydrogen bonded interactions in case of AZAC included, His22, Gly113, Asn116, and Ser148. As indicated by fittings of two ligands and the number of interactions made by them with protein atoms, AAtA appears to be a more compatible with the structure of the substrate binding cleft. However, there is a further scope to achieve a better stacking than that of O-tyrosyl moiety because it is not still ideally stacked. These observations about the interactions between the protein and ligands have provided the information about the mode of binding of ligands, nature and number of interactions. This information may be useful for the design of tight inhibitors of Pth enzymes.

Keywords : peptidyl tRNA hydrolase, Acinetobacter baumannii,Pth enzymes, O-tyrosyl
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