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Structural characterization of peptidyl-tRNA hydrolase from *Vibrio cholerae* and its mutants by X-ray crystallography and NMR spectroscopy

Salman Shahid
CSIR Central Drug Research Institute, India

Bacterial peptidyl-tRNA hydrolase (Pth; EC 3.1.1.29) is an essential enzyme that hydrolyses peptidyl-tRNAs accumulated in the cytoplasm due to ribosome stalling, minigene expression or antibiotic treatment. The enzyme spans the different binding regions for the peptide and tRNA moiety involving three asparagines (N14, N72 and N118) for the peptide binding and positive charged clamp (K107, K109 and R137) for the tRNA binding. The catalytic base histidine (H24) present on the crevice of the two sites. We have solved X-ray crystal structure of *Vibrio cholerae* Pth (VcPth) at two different pH, 7.5 and 6.5 with the bound citrate at resolution of 1.63 Å and 2.01 Å, respectively and the structure of its mutants N14D, H24N, N118D and K146A at 2.40Å, 2.43Å , 1.63Å and 1.63Å resolutions, respectively. In all the crystal structures, two protein molecules were observed in the asymmetric unit orienting in the manner that their active site are facing each other in the reciprocating manner, while the structure solved at the pH 6.5 has interacting C-terminal ends. This different orientation is most likely a result of pH difference, which allows the protein to pack in the different space group as observed in the crystal data. The crystal structure of the wild type VcPth accommodates a citrate ion, which is bonded to N14, H24, M71 and N118, residues at the active site groove. The citrate ion acts like a peptide bound to the active site and was confirmed by comparing the orientation of side chain and χ1 angle of the N118 with the corresponding residue in the other substrate bound structures like, *E. coli* Pth (EcPth) and *P. aeruginosa* Pth (PaPth). Among all the above mutants, only K146A was able to retain the citrate ion in the active site while all other mutants have lost it. The solution structure of the VcPth was also determined and chemical shift perturbations for H24N and N118D were mapped. In the 1H-15N HSQC spectrum of wt-VcPth, the amide correlation peak for N118 was not observed, but it was observed in the 1H-15N HSQC spectrum of H24N and N118D mutants. This suggests that the H24N mutation leads to changes in dynamics of the peptide binding region proximal to the site of catalysis. The pH dependent CSP analysis shows that protein undergoes conformational changes with the change in pH and the maximum effect was observed at the lid region of the protein. The complete unfolding of protein was observed at the pH 4.5.

Biography

Salman Shahid is currently a PhD student at CSIR-Central Drug Research Institute, India under the supervision of Dr. Ashish Arora, Senior Scientist, in the Structural Biology Laboratory. He was awarded with the Junior Research Fellowship (JRF), by the University Grant Commission (UGC), Government of India. He is working on protein, peptidyl-tRNA hydrolase and characterization of its mutants with the help of X-ray crystallography and NMR spectroscopy. He has submitted four structures in protein data bank, having IDs: 4ZXP, 4Y2Z, 4Z86 and 5EKT.

salmanshahid3@gmail.com

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