Stereochemical analysis of the active site of bacterial Peptidyl-tRNA hydrolase

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ABSTRACT

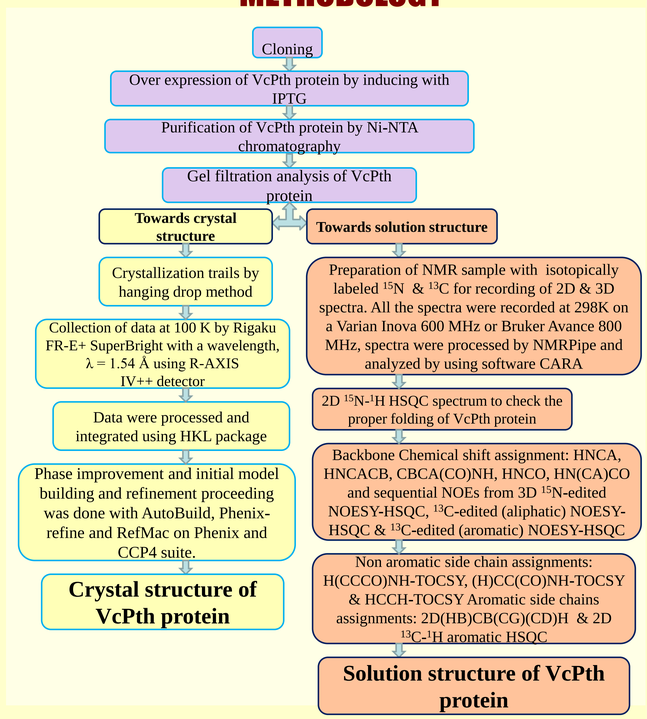
Bacterial peptidyl-tRNA hydrolase (Pth; EC 3.1.1.29) is an essential enzyme that hydrolyzes the peptidyl-tRNAs accumulated in the cytoplasm due to ribosome stalling, minigene expression or antibiotic treatment, and thereby prevents cell death by alleviating tRNA starvation. The critical base that catalyses the hydrolase activity is a histidine residue (H24) present on a crevice, which serves as the substrate binding site. Here we present the NMR solution and X-ray crystal structure at 1.63 Å resolution of Vibrio cholerae Pth (VcPth), and the structure of its H24N mutant at 2.43 Å resolution. Additionally, we have assigned the backbone chemical shifts for the H24N mutant, and for the wild-type protein at pH 5.2. Based on the NMR based chemical shift perturbation (CSP) studies, we have mapped the effects of the H24N mutation and pH on the conformation of VcPth. The H24N mutation affects the hydrogen bond network and the dynamics of the peptide binding region, while the lowering of pH mainly affects the catalytic site and lid regions. Further, through CSP studies, we have mapped the binding of puromycin to the wild-type VcPth. Our results indicate that the activity of Pth proteins is regulated through a series of hydrogen bonds involving the highly conserved residues H24, D97 and N118, and highlight a hidden interaction between H24 and N118.

INTRODUCTION

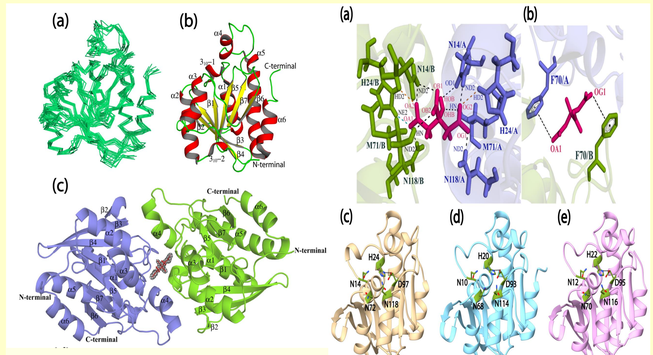
The process of protein translation is the target of several clinically used antimicrobial drugs. This process is aborted prematurely in 10% of cases due to ribosome stalling, and also because of minigenes expression and effect of macrolide antibiotics[1-2]. The stalling and subsequent disruption of the ribosome leads to accumulation of peptidyl-tRNA in the cytosol. This accumulation is toxic to the cells and also creates scarcity of free tRNA for further protein synthesis[3]. This condition is salvaged by an enzyme, peptidyl-tRNA hydrolase (Pth), which cleaves the ester linkage between the C-terminal carboxyl group of the peptide and the 2'- or 3'-hydroxyl of the ribose at the 3' end of tRNA, thus releasing free peptides and tRNA for reuse in protein synthesis[4-5]. Pth activity has been demonstrated to be essential for the viability of bacterial cells. Therefore, bacterial Pths are strong candidate for the development of antibacterial agents.

Figure: The catalytic action of peptidyl-tRNA hydrolase

METHODOLOGY

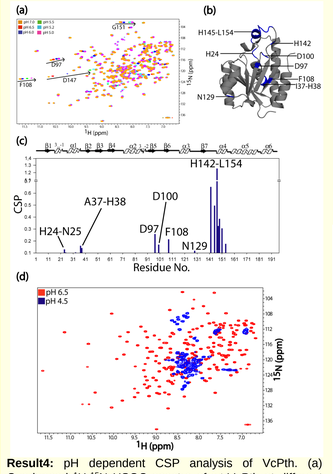


RESULTS



Result1: (a) the final ensemble of 10 solution structures of VcPth with lowest target function; (b) lowest energy structure of VcPth solution structure (2MJL). (c) the crystal structure of VcPth (PDB ID: 4ZXP) showing two protein molecules in crystallographic unit cell and citrate molecule is shown at interface of the two molecules.

Result2: (a) The citrate ion at intermolecular interface shown to have hydrogen bonds (black) with the active site residues. The salt-bridge in cyan dashes and non-conventional H-bonds in orange dashes; (b) The anion-π interaction between citrate and two chains of VcPth is shown in black dashes; (c), (d) and (e) Ribbon representation of wt-VcPth (PDB ID:4ZXP), EcPth (PDB ID:2PTH) and PaPth-AAtA (PDB ID:4QBK) showing the side chain orientation of some catalytically important residues as green sticks. A catalytically important H-bond between H24 and D97 is shown in black dashes. All these three structures show open gate conformation.



Result3: CSP analysis for H24N mutant of VcPth. (a) Overlapped ^{1}H-^{15}N HSQC spectra of wt-VcPth with H24N mutant. Red peaks represent for the wt-VcPth and blue peaks represent for the H24N mutant. Some residues, which show CSP > 0.13 ppm are labeled; (b) Residues, showing CSP > 0.13 ppm, are mapped on VcPth solution structure with blue color and labeled; and (c) Plot showing CSPs as a function of residue number. Residues, which show CSP > 0.13 ppm, are labeled.

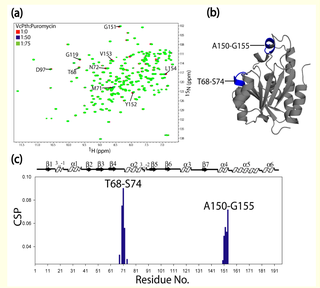
Parameter	Value
Total NOE upper distance limits	2730
Intra-residue {i=j}	506
Sequential {(i-j)=1)}	738
Medium range (1<{i-j}>5)	669
Long range ({i-j}>5)	817
Dihedral angle constraints	307
Hydrogen bonds	114
RMSD to mean coordinates	
Backbone heavy atoms (A)	0.8
All heavy atoms (A)	1.2
Secondary structural elements (A)	0.7
PROCHECK Ramachandran plot analysis:	
Most favoured region (%)	89.1%
Additional allowed region (%)	9.8%
Generously allowed region (%)	0.4%
Disallowed region (%)	0.6%
PDB ID	2MJL

Table1: Experimental NMR data and structural statistics for VcPth.

	wtVcPth	H24N
Cell parameters		
a, b, c (Å) α, β, γ (°)	44.718,73.628,124.201 90.0, 90.0, 90.0	44.569,72.6280,124.183 90.0, 90.0, 90.0
V_m (Å^3 Da^{-1})	2.38	2.36
[a]Resolution range (Å)	50.0-1.63 (1.69-1.63)	50.0-2.45 (2.54-2.45)
Average redundancy	11 (9.2)	5.3 (4.8)
Average I/ σ (I)	32.3 (3.2)	14.42 (2.38)
Completeness (%)	95.0 (89.5)	95.7 (97.8)
[b]Rmerge (%)	0.081(0.772)	0.115 (0.785)
Refinement and Structural Model		
[c]R factor (%)	17.1	19.3
Free R factor (%)	21.1	25.6
No. of atoms		
Protein	2992	2956
Water	331	121
Average B factor (Å^2)		
Overall	31.22	40.88
Ramachandran plot (%)		
Favored	95.1	94.5
Allowed	4.9	5.5
PDB ID	4ZXP	5B6J

Table2: Summary of Diffraction data and Structure Refinement Statistics.

Result4: pH dependent CSP analysis of VcPth. (a) Overlapped ^{1}H-^{15}N HSQC spectra of wt-VcPth at different pH. Color code for different pH as follows; Orange-7.0, Red-6.5, Blue-6.0, Green-5.5, Cyan-5.2 and magenta-5.0. Some residues showing high CSPs are labeled and the shift from high pH to low pH is denoted by an arrow for the corresponding residue; (b) Residues, showing CSP > 0.1 ppm, are mapped on VcPth solution structure with blue color and labeled; (c) Plot showing CSPs as a function of residue number. Residues, which show CSP > 0.1 ppm, are labeled; and (d) Overlapped ^{1}H-^{15}N HSQC spectra of wt-VcPth at pH 6.5 and pH 4.5 showing unfolding of the protein at pH 4.5. Red peaks represent for the wt-VcPth at pH 6.5 and blue peaks represent for the wt-VcPth at pH 4.5



Result5:. CSP analysis of VcPth with puromycin binding. (a) Overlapped ^{1}H-^{15}N HSQC spectra of wt-VcPth at different concentrations of puromycin. Color code for different protein: puromycin ratios as follows; orange-1:0, Blue-1:50 and green-1:75. Some residues, which show significant CSPs (> 0.025 ppm), are labeled; (b) Residues, showing CSP > 0.025 ppm, are mapped on VcPth solution structure with blue color and labeled; and (c) Plot showing CSPs as a function of residue number. Residues, which show CSP > 0.025 ppm, are labeled

DISCUSSION

- ❖ The overall structure of the VcPth is comparable to the other reported structures of bacterial Pth.
- ❖ In comparison with the crystal structures of *E. coli* Pth and *P. aeruginosa* Pth, the VcPth crystal structure represents the 'peptide filled-like', 'open gate' conformation, as opposed to the 'peptide filled-like', 'closed gate' conformation observed in *M. tuberculosis* Pth, and 'peptide empty-like', 'closed gate' conformation observed in *S. pyogenes* Pth.
- ❖ The H-bond between H24 and D97, which is conserved in all other canonical Pth structures, is lost in the H24N mutant structure of VcPth.
- ❖ The amide correlation peak for N118 was observed in the ^{1}H-^{15}N HSQC spectrum of H24N mutant, while it was not observed in the wild-type protein. This suggests that the H24N mutation leads to change in dynamics of the peptide binding region proximal to the site of catalysis.
- ❖ pH mainly affects the catalytic site and lid regions and N118 could again be assigned at pH 5.2, which reflects the pH induced change in dynamics of N118.
- ❖ Puromycin does not lead to the appearance of N118 even at a molar ratio of 1:75, indicates weak binding or binding mode that does not affect the D97-H24-N118 interaction

CONCLUSIONS

In the structure of VcPth, the H24-D97 interaction is most important for catalysis. H24N mutation and pH titration study reveals the change in dynamic behavior of N118 due to change in H-bonding network between these residues. Conformational changes induced by pH are expected to be similar to those induced by substrate binding. Overall, the titration studies and structure of VcPth and their relevant comparison to other Pth proteins, especially *M. tuberculosis* Pth, has significantly improved the understanding of hydrogen bonding networks and related dynamics operating in the structural segments important for the catalysis.

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