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 hydrolysis 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. 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. 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. Pth activity has been demonstrated to be essential for the viability of bacterial cells. Therefore, bacterial Pths are strong candidates for the development of antibacterial agents.

METHODOLGY

Cloning

Preparation of VcPth protein by Ni-NTA chromatography

Crystallization trials by hanging drop method

Data collection at 1.63 Å by Rigaku FR-E i SuperBright with a wavelength, λ = 1.2 Å using R-Axis IV+ detector

More protein and reagent model building and refinement processing done with AutoBuild, PhireMut and Refmac on Protein and CCP4 suite.

Crystal structure of VcPth protein

RESULTS

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 1H-13N 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 related 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.

REFERENCES


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