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Review

Structural and functional insights into peptidyl-tRNA hydrolase

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ABSTRACT

Peptidyl-tRNA hydrolase is an essential enzyme which acts as one of the rescue factors of the stalled ribosomes. It is an esterase that hydrolyzes the ester bond in the peptidyl-tRNA molecules, which are products of ribosome stalling. This enzyme is required for rapid clearing of the peptidyl-tRNAs, the accumulation of which in the cell leads to cell death. Over the recent years, it has been heralded as an attractive drug target for antimicrobial therapeutics. Two distinct classes of peptidyl-tRNA hydrolase, Pth and Pth2, have been identified in nature. This review gives an overview of the structural and functional aspects of Pth, along with its sequence and structural comparison among various species of bacteria. While the mode of binding of the substrate to Pth and the mechanism of hydrolysis are still speculated upon, the structure-based drug design using this protein as the target is still largely unexplored. This review focuses on the structural features of Pth, giving a direction to structure-based drug design on this protein.

1. Introduction

Protein synthesis is a well programmed activity of the cell which occurs in the ribosomes. It starts with the aminoacyl tRNA, entering the ribosome at the aminoacyl site (A-site) followed by the formation of peptidyl-tRNA, which binds to the peptidyl site (P-site) and finally, the free tRNA exits the ribosome at the exit site (E-site) after giving its amino acid to the nascent peptide chain. However, at times, this process comes to a premature halt. This sudden halting of the translation of genetic information from mRNA to protein chain is known as Ribosome Stalling [1,2].

Ribosome Stalling can be due to various factors such as defective or truncated mRNAs [3], amino acid starvation [4] or tRNA starvation [5]. The result of a stalled ribosome is the release of peptidyl-tRNAs, which are intermediates in the process of protein synthesis. The production of peptidyl-tRNAs is highly toxic to the cell [6–8]. Since these stalled ribosomes are bound to be lethal for the cell, they have to be rescued. In order to protect the cell, there exists a ribosome rescue taskforce within the cell which comprises of several varying factors such as some proteins, the most significant example being peptidyl-tRNA hydrolase (Pth); tm-RNA [9]; protein synthesis factors, such as release factors (RF1, RF2, and RF3); ribosome recycling factor (RRF) [10]; alternative ribosome rescue factors [11] and elongation factors [12].

Peptidyl-tRNA hydrolase (Pth) (E.C.3.1.1.29) is an esterase which is responsible for the hydrolysis of the ester bond between the peptide and the ribose of the tRNA [13,14]. The ester bond on which it acts exists between the carboxy-terminal end of the peptide and the 2′- or 3′-hydroxyl of the ribose at the end of the tRNA. The final result of this reaction is free tRNA which can be recycled or reused for protein synthesis again. Pth activity has been determined to be vital for the survival of bacterial cell [7,8].

While Pth activity was first identified in *Escherichia coli* [14] and yeast [15], it has been observed over the years that Pth activity is ubiquitous in nature. The *pth* gene in *E. coli* was first identified in *E. coli* in 1991 [16]. The genes encoding Pth were identified in both bacterial [17] and eukaryotic kingdoms [18] but were not found in archaea. On the other hand, Pth-like activity was detected in some archaea, for example, *Methanocaldococcus jannaschii* and *Sulfolobus solfataricus* [18,19]. This new class of Pth, named as Pth2, did not show significant sequence homology with the Pth enzymes [20,21]. However, the functions of both Pth and Pth2 are similar, as was evident when it was demonstrated that *E. coli* strains which do not contain endogenous Pth expression, can survive with the help of Pth2 expression [18]. It was assumed that while Pth and Pth2 exclusively belong to bacterial and archaeal kingdoms respectively, eukaryotes contain various other types of Pths apart from orthologs of Pth and Pth2 [18]. In eukaryotes, Pth2 may be localized in the mitochondria and may be involved in mitochondrial protein synthesis [18]. However, later it was speculated that Pth2 may be present in archaeal, bacterial and eukaryote families [22]. Though it was assumed that Pth and Pth2 are essential enzymes in bacteria [7,8], it was observed that orthologs of Pth and Pth2 orthologs are non-essential in yeast [18].

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2. Comparison of structural features of Pth and Pth2

Among Pth, the three-dimensional crystal structures of Pth from *E. coli* [21], *Mycobacterium tuberculosis* [23], *Francisella tularensis* [24], *Mycobacterium smegmatis* [25], *Pseudomonas aeruginosa* [26,27], and *Acinetobacter baumannii* [28] have been determined. Among Pth2 class of enzymes, the crystal structures of human Pth2 [20] and Pth2 from archae *S. solfataricus* [29] and *Pyrococcus horikoshii* OT3 [30] have been determined. Both the classes of enzymes, Pth and Pth2, show large variation in terms of sequence and structural features. The sequence comparison of Pth from *A. baumannii* (*Ab*Pth) and human Pth2 did not reveal a significant sequence identity.

Structurally, Pth is a monomeric, globular molecule containing 191–194 residues, adopting an α/β fold, spanning about 41 Å in length and 35 Å in width. The compact, globular molecule contains six α-helices [α1–α6] and seven β-strands [β1–β7] which are conserved in all the species (Fig. 1a). The core of the molecule is made up of a twisted mixed β-sheet, surrounded by six α-helices. The β-strands, β1, β3, β4, β5 and β7 form the center molecule with β2 and β6 flanking the center from both sides. The central core is enclosed by the six α-helices in such a way that the two long helices, α1 and α5 and one short helix, α6 are stacked on one side, while helices α2 and α3 are stacked on the other side. The remaining short helix, α4, forms the roof of the molecule and also contributes to the architecture of the substrate binding site (Fig. 1b). On the other hand, Pth2 is shorter in length (116 amino acids) and adopts an α/β fold. The structure of Pth2 comprises of four α-helices [α1–α4] and four β-strands [β1–β4]. The four β-strands form a twisted β-sheet in the center of the molecule which is flanked by the four α-helices (Fig. 2a). The Pth2 molecule is significantly more compact as compared to Pth, with its dimensions being 34 Å in length and 25 Å in width (Fig. 2b).

3. Pth: sequence and structure variations across bacterial species

The sequence comparison of Pth from *A. baumannii* (*Ab*Pth) with Pth from *E. coli* (*Ec*Pth), *P. aeruginosa* (*Pa*Pth), *F. tularensis* (*Ft*Pth), *M. tuberculosis* (*Mt*Pth) and *M. smegmatis* (*Ms*Pth) revealed sequence identities of 53%, 58%, 43%, 39% and 41% respectively (Fig. 3).

The three active site residues, His22, Asp95 and Asn116 [numbering according to *Ab*Pth] are conserved in all the species. It was observed that there are two major sites in Pth which have a significant role in substrate recognition; the first site clamps the 5′-phosphate of elongator peptidyl-tRNAs while the second site holds the 3′-end of the peptidyl-tRNA [31,32]. The two residues which have been identified to play a role in the first site are Lys105 and Arg133. It was noted that while Arg133 is strictly conserved in all species, Lys105 is either conserved or substituted to a homologous arginine residue. The second site, conserved in Pths of all the species, consists of an asparagine cluster which includes four asparagine residues, Asn10, Asn21, Asn68 and Asn114.

The dimensions of the Pth molecule were determined by measuring the distances between Gly140 Cα and Tyr83 Cα in one direction and Gln182 Cα and Thr67 Cα in the other direction [*Ab*Pth numbering scheme]. The dimensions of the Pth molecule were found to be 41 Å × 34 Å in *Ab*Pth, 41 Å × 35 Å in *Ec*Pth, *Ft*Pth and *Pa*Pth, 43 Å × 35 Å in *Ms*Pth and 43 Å × 34 Å in *Mt*Pth respectively.

Upon superimposition of the Cα traces of all the Pths, the overall structures of all the proteins were found to be very similar. However, the r.m.s. shifts of *Ab*Pth with the other Pths showed an interesting pattern. The r.m.s. shifts of the structure of *Ab*Pth were found to be 0.7 Å with *Ec*Pth, 0.7 Å with *Pa*Pth, 0.9 Å with *Ft*Pth, 1.5 Å with *Ms*Pth and 1.4 Å with *Mt*Pth. The molecular dimensions of various Pth molecules indicate that while the dimensions of Pths from different species of bacteria have similar values, *Ab*Pth, *Pa*Pth, *Ft*Pth and *Ec*Pth are somewhat more compact as compared to *Ms*Pth and *Mt*Pth. Also, the relatively larger r.m.s. shifts of *Ms*Pth and *Mt*Pth with other Pths validated the fact that *Ms*Pth and *Mt*Pth structures show more diversion from the other Pth structures.

The major regions where all the Pth molecules were different from each other were the N-terminal stretch, Met1–Ser5, the C-terminal stretch of Val180–Ala193 which contains α6 region, the α2 region Arg71–Lys81, the α3 region of Gly114–Val122, the region of His138–Val149 which contains a loop and α4 and finally the loop Pro123–Phe129. While it is possible that the N-terminal and the C-terminal stretches could be different due to being disordered, the other regions show differences that could be significant to the function of the protein.

In two significant studies involving crystallographic analysis and NMR mapping of the complexes of Pth with RNA domains, it was noted that the tRNA binding sites in Pth were limited to some specific sites within the molecule [32,33]. Giorgi et al. observed that the

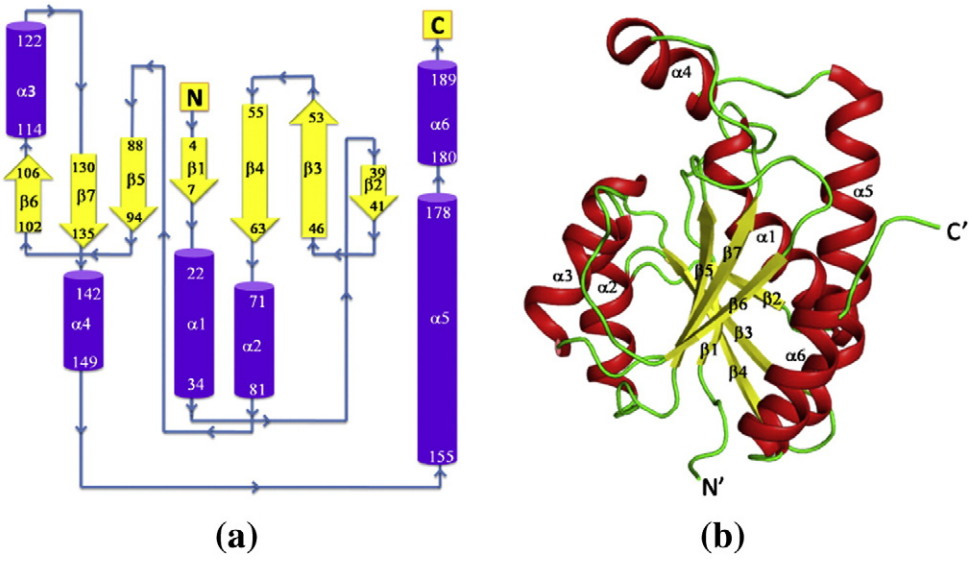

Fig. 1. (a) Topological diagram of the secondary structure of *Ab*Pth from *Acinetobacter baumanni* showing the spatial arrangement of α-helices and β-strands. The beginning and the end residues of each secondary structure element have been indicated. (b) Cartoon diagram of the structure of *Ab*Pth from *A. baumanni*. The α-helices and β-strands are labeled. The N- and the C-termini are indicated.