

Editorial

Recent Antimicrobial Developments Targeting Peptidyl-tRNA Hydrolases

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Abstract

Peptidyl-tRNA hydrolases (Pths) are essential enzymes found in bacteria, archaea, and eukaryotes. Pths cleave the peptide:tRNA ester bond of peptidyl-tRNAs generated from premature termination of protein synthesis and the expression of short ORFs or minigenes. Accumulation of peptidyl-tRNAs is toxic presumably due to impaired translational initiation or slowed protein synthesis caused by specific tRNA starvation. Pth activity is thus vital for cells to deal with the build-up of peptidyl-tRNAs.

Bacterial genomes encode a highly conserved peptidyl-tRNA hydrolase enzyme, Pth1. Amino acid sequence homology is high across all species and active site residues are strictly conserved. Structures of Pth1 have been solved for several species and catalytically important residues identified. Studies with mini- and partial-substrates have contributed to understanding of substrate recognition. However, the mechanism of action and ability to distinguish between aminoacyl- and peptidyl-tRNAs are not fully understood.

Better understanding of this fundamental biological enzyme has contributed to pharmacological development against this promising new antibacterial target. Expediting the time consuming northern blot assay has paved the way for moderate screening efforts and identification of inhibitory natural product extracts. Computational small molecule docking has also provided initial insight. Overall, Pth1 is a promising new antibacterial target and continued characterization will advance antibiotic development.

INTRODUCTION

Pth Activity is essential and Ubiquitous

Peptidyl-tRNAs are generated when ribosomes abort translation prematurely [1-3], which occurs on average 10% of the time [4]. Peptidyl-tRNAs are then released by ribosome recycling factor and elongation factor-G [4,5], are rescued (i.e. by tmRNA [6] or YaeJ [7,8]), or fall-off at a rate depending on the attached tRNA [9]. Build-up of peptidyl-tRNAs results in tRNA starvation and rapid cell death. Accumulation of peptidyl-tRNAs also results from the expression of minigenes or short ORFs [10-12]. To avoid excessive build-up of polluting peptidyl-tRNA, it is vital for cells to maintain peptidyl-tRNA hydrolase activity.

Pth activity is universal throughout all kingdoms of life. However, unlike the highly conserved Pth enzymes in bacteria, referred to as Pth1 enzymes, multiple Pths (Pth1, Pth2, and Pth-like domains) are found in archaea and eukaryotes. There is no sequence or structural homology between Pth1 and Pth2 enzymes and the mechanism of bacterial Pth1 action has been demonstrated to be completely different from that of Pth2

[13]. In rabbit reticulocytes, peptidyl-AMP and tRNA-CC are the products of the reaction [14] not just tRNA and peptide. Also, the ability to cleave bacterial initiator fmet-tRNA^{Met} was found in reticulocytes, a property absent in bacterial Pth1s [15,16].

Structures of Pth1 and Pth2 enzymes have been solved. Focusing on Pth1, the crystal structure of the 21 kDa *E. coli* Pth1 monomer was solved to 1.2 Å [17]. More recently, crystal and solution structures for *M. tuberculosis* Pth1 have been reported [18,19] along with Pth1 from other pathogenic species including *P. aeruginosa* [20], *F. tularensis* [21], *M. smegmatis* [22], and *A. baumannii* [23]. As predicted from the high degree of amino acid sequence similarity, all have the same backbone fold. Pth1 family members are globular, single domain proteins that have a central mixed β -sheet surrounded by α -helices (Figure 1). Helix-4 occludes three residues, N10, H20, and D93 (as numbered in *E. coli* Pth1), identified by site directed mutagenesis to be crucially involved in enzyme activity [24]. ¹⁵N NMR relaxation data suggest the putative active site and the helix-4 cover exhibit motions on the millisecond to microsecond timescale, thought to be linked to interaction with the peptidyl-tRNA substrate [18].

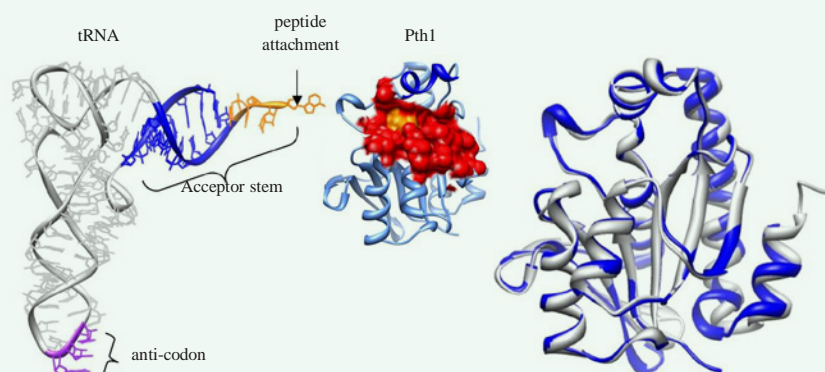


Figure 1 Bacterial Peptidyl-tRNA Hydrolases.

Left: tRNA^{Phe} (1EHZ) is shown next to *P. aeruginosa* Pth1 (4FYJ). The substrate binding site of Pth1 is shown as a red surface with catalytically important His20 in yellow. Helix-4 is in dark blue. The unpaired region of the tRNA acceptor stem is shown in orange. Right: Ribbon diagram of Pth1 from *P. aeruginosa* (blue, 4FYJ) aligned with *E. coli* (white, 2PTH). Heavy backbone atom RMSD is 0.63 Å.

Speculation on the enzymatic mechanism include H₂O acting as a catalytic base. Residues K105 and R133 are suggested to be involved in clamping the 5'-phosphate group of the tRNA moiety [17,24-26]. Structural information is needed to resolve these postulates, yet no structure of the enzyme-substrate complex has been determined for any Pth.

Pth1 is a Promising Target for Antibacterial Development

With the relentless development of drug resistance and re-emergence of many pathogenic bacteria, the need for new antibiotics and new antibiotic targets is urgent and growing [27,28]. It is well known that the number of antimicrobial agents being brought to market has steadily declined [29]. Pth1 provides a much needed avenue for novel antibacterial development. The essential function, high conservation across bacterial species, and lack of essential human equivalent make Pth1 a prime target for antibacterial development. The high amino acid sequence conservation across bacterial species suggests a small molecule inhibitor found against one is likely to work against many others. Thus, broad spectrum inhibitors targeting Pth1 are a distinct possibility.

Targeting a new essential bacterial enzyme, Pth1 inhibitors will be effective against drug resistant strains and provide the potential for fresh, new, and original combinatorial therapies. Although bacteria have but a single class of Pth enzyme, eukaryotes possess several structurally unrelated Pth enzymes that subsume peptidyl-tRNA hydrolase activity. Pth1 knock out does not alter cell viability in yeast [30], thus Pth1 does not appear to be essential in eukaryotes. The very different structure for Pth2 [13,31-33] and Pth-domain containing proteins [34,35] mitigates concern for mitochondrial Pth impairment.

Molecular modeling studies indicate two proximal binding sites for small molecules on either side of the catalytically important H₂O residue, in the crevice containing the putative active site [18]. The next step in targeted inhibition is already underway. Natural product screening shows promise for revealing Pth1 specific inhibitors [36,37]. Also, screening of

a custom combinatorial library has also revealed a family of compounds that show potent *in vitro* and *in vivo* inhibition. Both show that Pth1 specific inhibition is possible, not inhibiting Pth2 *in vitro*.

CONCLUDING REMARKS

The solid structural biology foundation and first identification of specific small molecule inhibition of Pth1 are beginning to bridge the gap between *in vitro* biochemical studies and lead compound identification. Such translational progress will continue, aided by medicinal chemistry and computational studies further contributing to small molecule binding studies. Overall, Pth1 is a promising new antibacterial target and comprehensive characterization will significantly advance structural biology of the system, understanding of the Pth:peptidyl-tRNA interaction, and possibly development of next generation antibiotics.

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