Synopsis

Elucidating the structural basis of the editing mechanism in an archaeal aminoacyl-tRNA synthetase.

Aminoacyl-tRNA synthetases (aaRSs) govern the fidelity of translation of the genetic code by attaching the correct amino acid to the corresponding tRNA. However, mistakes are often made if aaRSs have to discriminate between structurally similar amino acids like isoleucine/leucine/valine or threonine/serine/valine leading to mischarging of tRNA by noncognate amino acid. Such aaRSs possess editing domains to hydrolyze the mischarged tRNA. Editing of noncognate amino acids is essential because aaRSs that are editing-defective form statistical proteins with incorrect sequences leading to misfolding of proteins and hence are linked to various diseases including neurodegeneration. This underlines the biological relevance of editing activity and presents an important fact that the accurate selection of cognate amino acid as well as the discrimination of noncognate amino acid differing by a methyl group is a fundamental chemistry problem during translation of the genetic code.

To ensure a high fidelity during translation, threonyl-tRNA synthetases (ThrRSs) harbor an editing domain that specifically removes noncognate L-serine attached to tRNA<sup>Thr</sup>. Most archaeal ThrRSs possess a unique editing domain structurally similar to D-aminoacyl-tRNA deacylases (DTDs) found in eubacteria and eukaryotes that specifically removes D-amino acids attached to tRNAs. It would be interesting to know how a DTD-like fold functions in archaeal ThrRS to specifically remove noncognate L-Ser and not cognate L-Thr from tRNA<sup>Thr</sup> whereas DTDs have evolved to remove chemically diverse D-amino acids attached to tRNA. This forms the objective of the thesis and x-ray crystallography duly complemented with biophysical, biochemical and mutational studies have been used for the mechanistic details. The work carried out elucidates the structural basis of editing mechanism in the editing domain of *Pyrococcus abyssi* ThrRS (Pab-NTD) that has been summarized below.
Chapter 1: Introduction: Fidelity in translation of the genetic code

This chapter briefly describes aaRS family of enzymes that is followed by a detailed review of all editing domains and their mechanism of action. The biological relevance of editing activity is presented along with the need to understand the different editing strategies that nature has evolved to remove noncognate amino acids from tRNAs. The objectives and the approach of the thesis work are also mentioned after providing a detailed description of the earlier work on the unique editing domain of archaeal ThrRS.

Chapter 2: Materials and Methods

This chapter deals with the materials and methods used for the experiments that are discussed in this thesis work. Each technique (Fluorescence Spectroscopy, Isothermal Titration Calorimetry and NMR Spectroscopy) used is briefly described with a major emphasis on x-ray crystallography. The details of protein purification, crystallization, structure determination and refinement are mentioned in this chapter and the same strategy was followed for solving all the structures of Pab-NTD complexes mentioned in this study. The deacylation assay used for the biochemical activity of Pab-NTD and its mutants is also mentioned in this chapter.

Chapter 3: Substrate-assisted catalysis to remove mischarged L-Ser-tRNA<sub>Thr</sub>

In order to elucidate the structural basis of the editing mechanism of the noncognate L-serine removal from tRNA<sub>Thr</sub> in archaeal ThrRS, high-resolution crystal structures of Pab-NTD with substrate analog mimicking mischarged L-Ser-tRNA<sub>Thr</sub> were determined. Based on structural and sequence analysis, mutations were designed and the Pab-NTD mutants along with native protein were tested for the editing activity against L-Ser-tRNA<sub>Thr</sub>. Together, the structural analysis complemented with the mutational and biochemical studies of Pab-NTD elucidate the editing mechanism of the L-Ser-tRNA<sub>Thr</sub> that is presented in this chapter.
Chapter 4: Non-productive binding of aminoacyl-adenylates rules out pre-transfer editing

Pre-transfer editing has been a subject of debate in recent times for its physiological relevance and mechanistic details. This chapter introduces this debate and provides a clear picture of pre-transfer editing in archaeal ThrRS. Pab-NTD complexes with substrate analogs mimicking pre-transfer substrates (aminoacyl-adenylates) solved at moderate to high resolution are presented. The structural analysis unambiguously rules out pre-transfer editing in Pab-NTD since a non-productive mode of binding was observed for all aminoacyl-adenylate analogs without any selectivity for the side chain.

Chapter 5: Revisiting the ‘Double-Sieve Model’ for editing mechanism in aaRSs

This chapter deals with the discrimination of the cognate aminoacyl-tRNA, differing by a methyl group, at the editing site. This rejection has been mainly attributed to steric hindrance adhering to Alan Fersht’s ‘Double-Sieve Model’. This chapter revisits the ‘Double-Sieve Model’ and presents detailed solution studies and high resolution crystal structures of Pab-NTD with substrate analog mimicking cognate L-Thr-tRNA\textsuperscript{Thr}, bringing out the exact mode of rejection of cognate L-Thr-tRNA\textsuperscript{Thr} in Pab-NTD. The chapter provides mechanistic insight into the mode of discrimination of cognate aminoacyl-tRNA at the editing site, adding a new perspective to the proofreading mechanism.

Chapter 6: A structural model for editing-defective aminoacyl-tRNA synthetases

A mutation in the editing domain that compromises the editing activity leads to disease conditions including neurodegeneration. This chapter deals with the biochemical, biophysical and structural study of a Pab-NTD mutant that was selected based on certain criteria. The structure of the mutant with and without substrate analogs provides, for the first time, a structural model of editing-defective aaRSs that is defective in the deacylation activity of mischarged L-Ser-tRNA\textsuperscript{Thr} as well as in the discrimination of correctly charged L-Thr-tRNA\textsuperscript{Thr}.
Chapter 7: Structural and functional homology of Pab-NTD with D-aminoacyl-tRNA deacylases

This chapter brings forth the structural picture of the stereoselectivity of Pab-NTD. Complexes of Pab-NTD and its mutant with a substrate analog, mimicking D-Ser-tRNA, elucidate the recognition mode of opposite chirality (D-aminoacyl moiety) in this DTD-like fold. The structural analysis provided by Pab-NTD complexes were used to gain mechanistic insights into the editing mechanism of DTDs that share structural homology with Pab-NTD.

Chapter 8: Conclusions and Future directions

This chapter sums up the major conclusions drawn from the study. Also, the probable impact of the work on the field of aaRS editing is mentioned, namely tRNA-mediated ‘substrate-assisted’ post-transfer editing mechanism, revised ‘Double-Sieve Model’, structural model for editing-defective aaRS and enantioselectivity of editing domains. Furthermore, the future directions of research that may be looked into are discussed.

Overall, the study answers important questions on the editing mechanism as well as the design of nature with respect to how a D-aminoacyl-tRNA deacylase-like fold is utilized in the context of archaeal ThrRSs to remove L-Ser (opposite chiral molecule) mischarged on tRNA

Thr and paves the way for further detailed mechanistic studies on these editing modules.
List of publications


2. Mechanistic insights into cognate substrate discrimination during proofreading in translation; Tanweer Hussain et al., *(manuscript under communication).*

3. Structural model for editing-defective aminoacyl-tRNA synthetases; Tanweer Hussain et al., *(manuscript under preparation).*