Abstract

Glutamyl-queuosine-tRNA^Asp Synthetase (Glu-Q-RS) is a paralog of Glutamyl-tRNA synthetase (GluRS) and is present in the genomes of less than forty species of proteobacteria, cyanobacteria, and actinobacteria. Glu-Q-RS shows striking structural similarity with N-terminal catalytic domain of GluRS (NGluRS) but it lacks the C-terminal anticodon binding domain. In spite of structural similarities, they differ in their functional properties. One of the major functional differences is that Glu activation step of GluRS is tRNA dependent, whereas Glu-Q-RS can activate Glu independent of the presence of the tRNA. Another significant dissimilarity is that GluRS transfers the activated Glu to the 3’ accepting end of the cognate tRNA^Glu, on the contrary Glu-Q-RS transfers the activated Glu to the Q34 which is located in the anticodon loop of the tRNA^Asp.

Since NGluRS is able to catalyze aminoacylation of only tRNA^Glu the glutamylation capacity of tRNA^Asp by Glu-Q-RS is surprising. To understand the substrate specificity of Glu-Q-RS we undertook a systemic approach by investigating the biophysical and biochemical properties of the NGluRS (1–301), CGluRS (314–471) and Glu-Q-RS-CGluRS, (1–298 of Glu-Q-RS fused to 314–471 from GluRS). Circular dichroism, fluorescence spectroscopy and differential scanning calorimetry analyses revealed absence of communication between N-terminal domain (1–298 of Glu-Q-RS) and C-terminal domain (314–471 from GluRS) in chimera, in contrast to the native full length GluRS. The chimeric Glu-Q-RS is still able to aminoacylate tRNA^Asp but has also the capacity to bind tRNA^Glu. However the chimeric protein is unable to aminoacylate tRNA^Glu probably as a consequence of the lack of domain–domain communication.

*E. coli* Glu-Q-RS and GluRS both have zinc residue in their acceptor stem binding domain. Zinc plays a major role in amino acid discrimination and structural stability in aminoacyl tRNA synthetases. It is reported that zinc plays a crucial role in aminoacylation and structural stability in *E. coli* GluRS. Glu-Q-RS shows structural similarity with catalytic core of GluRS and phylogenetic analysis shows that Glu-Q-
RS and GluRS possess evolutionary relationship but the role of zinc in Glu-Q-RS is still unknown. To elucidate the role of zinc in *E. coli* Glu-Q-RS, we have constructed C101S/C103S Glu-Q-RS variant. Energy dispersive X-ray fluorescence show that the zinc ion still remained coordinated but the variant became structurally labile and acquired aggregation capacity. The extent of aggregation of the protein is significantly decreased in presence of the small substrates and more particularly by adenosine triphosphate (ATP). Addition of zinc increased the solubility of the variant significantly. The aminoacylation assay reveals a decrease in activity of the variant even after addition of zinc as compared to the wild-type, although the secondary structure of the protein is not altered as shown by the Fourier transform infrared spectroscopy study.

Despite their significant structural similarities, Glu-Q-RS and GluRS, shows distinct differences in L-Glu activation and substrate (ATP, tRNA) induced conformational changes. Thus, GluRS and Glu-Q-RS may provide a model example to study the effect of substrates on the protein structure. The transiently formed enzyme:ligands complex can lead to local or global conformational change to enzyme. By studying local or global conformational changes of enzymes upon ligand binding, we can compare ligand induced conformational changes of Glu-Q-RS with structurally similar NGluRS. The structural changes in the presence of the substrates can indicate the differences between substrate induced conformational changes of Glu-Q-RS and NGluRS. To explore the differences of substrate induced conformational fluctuations of Glu-Q-RS and NGluRS, a detailed understanding of the binding thermodynamics is required. Substrate induced conformational analysis can give us a clue to understand the structural basis of the distinct functional properties of these two evolutionary linked enzymes. Proteolytic studies and differential scanning calorimetry studies show that ATP has significant effect on the structure of NGluRS and Glu-Q-RS. Binding studies exhibit that NGluRS fails to recognize L-Glu selectively in the presence of ATP.
Similarly tRNA$^{Asp}$ as a large ligand induces significant conformational changes in Glu-Q-RS, however, tRNA$^{Asp}$ bound Glu-Q-RS is unable to recognize L-Glu specifically.