RIBOSOME AND ASSOCIATED CHAPERONES: STUDIES WITH ANTIBIOTICS AND PROTEIN FOLDING INTERMEDIATES

Abstract:
Ribosome is the cellular protein synthesizing machinery. Nascent proteins require the assistance of molecular chaperones, like DnaK-J-E and Trigger factor to ensure accurate folding into their biologically active form and to avoid misfolding and aggregation in crowded cytosol. It has been reported that the ribosome itself has chaperoning abilities and is capable of assisting in folding of proteins. The chaperoning activity of the ribosome originates in the domain V of the 23S ribosomal RNA of 50S subunit. The peptidyl transferase center (PTC) of the E. coli ribosome also resides in this rRNA domain. The ribosome bound unfolded protein is release in a folding competent state and the ribosome dissociates into subunits. The studies performed in the thesis is aimed at understanding a) the effect of ribosomal chaperone on the process of protein folding and aggregation and b) the mechanism of unfolded protein mediated subunit dissociation.

The partially folded forms of protein are aggregation prone and likely to be encounter with the ribosome as first chaperone. Our studies shows that the ribosome can bind to partially folded intermediates of bovine carbonic anhydrase II (BCAII) and lysozyme and suppress aggregation during their refolding. Studies were also performed with the in vitro transcribed domain V RNA and its variants. Comparative studies were performed in presence of chaperones DnaK and Trigger factor. In the final step of bacterial protein synthesis, the ribosome dissociate into subunits - 30S and 50S by a combine action of translation factors. Our studies demonstrate that the unfolded protein can act as an antiassociation factor for the 50S subunit. Ribosome interacting antibiotics can inhibit the dissociation process. The dissociation process could be occurred independently of ribosome’s chaperoning function.

In addition, our study reveals the tendency of ribosome to coaggregate with disulfide (lysozyme) and non-disulfide (BCAII) containing proteins under conditions that facilitate aggregation of the proteins. Under similar conditions isolated ribosomal RNA can also stimulates protein aggregation. The ability to initiate aggregation of ribosomal components might also be a reason underlying the toxicity of cellular protein aggregation.