Comparison of chaperone activities on refolding of aggregation prone protein folding intermediates

Ref: Project no: BT(Estt)RD-6/2011
Dr. Chandana Barat, Dept. of Biotechnology, St. Xaviers College, Kolkata

Protein aggregation is principally driven by hydrophobic interactions between their partially structured folding intermediates. Molecular chaperones that are capable of interacting with these intermediate states and improving their solubility and reactivation yields can be used as tools to address the biochemical nature of the folding and aggregation process. Ribosome the cellular translation apparatus can assist in protein folding with its chaperoning ability residing in the domain V of its 23S rRNA. The objectives of the proposed study were to study effect of the ribosome, its domain V RNA and ribosome associated chaperones like Trigger factor and the DnaKJE system on a) reactivation and b) aggregation process of the partially folded molten globule state of carbonic anhydrase II from both bovine (BCAII-m) and humans (HCAII). Our present studies show that the E.coli ribosome can assist in refolding from different unfolded forms of BCAII (Figure 1A). The ability of ribosome to assist folding of BCAII-m was completely suppressed in presence of antibiotic Blasticidin thus indicating that the process is domain V mediated (Figue 1B). The domain V RNA itself could also significantly improve the reactivation yields from BCAII-m at low concentration (0.3 uM), which could be completely inhibited in presence of domain V specific antibiotic chloramphenicol (Fig 2). We have also initiated with mutants of domain V RNA to elucidate the mechanism of its chaperoning function. Similar studies with a mutant (H107Y) of the human carbonic anhydrase that forms toxic aggregates and is implicated in the disease marble brain syndrome shall also be initiated. The fact that aggregation-prone proteins can form a) compact insoluble fibrils leading to protein conformational diseases or b) amorphous aggregates leading to formation of inclusion bodies that is a major obstacle in large scale industrial production of heterologous proteins, makes our studies relevant to both Industrial and Medical Biotechnology.

Figure 1:

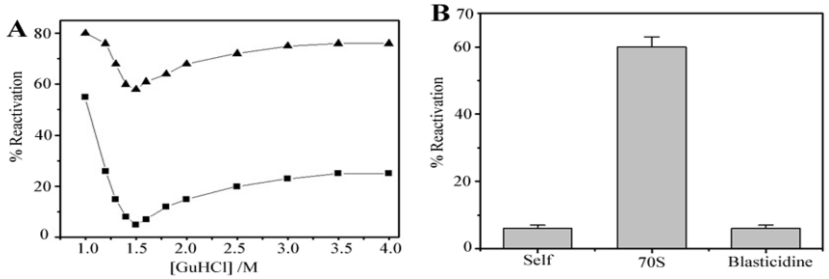

Figure 1 (A) Refolding yield of the proteins after incubation in various Guanidinehydrochloride concentrations. Symbols: (■) BCA II; (▲) BCA II in presence of E.coli 70S ribosome. B) Comparison of the net refolding of carbonic anhydrase after 30 minutes of refolding in absence of the folding modulator (self) and in presence of E.coli 70S ribosome and the antibiotic Blasticidin bound 70S ribosome as folding modulators.

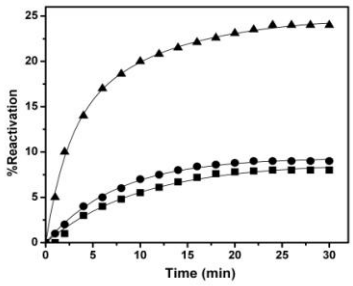

Figure 2: Time courses of reactivation of BCA II-m in absence of a folding modulator (■); presence of domain V RNA (▲); chloramphenicol bound domain V RNA (●).