Introduction-

GrpE, a molecular co-chaperone, along with the DnaK co-chaperone assist the DnaK molecular chaperone in various protein folding, protein assembly and disassembly processes. GrpE also referred to as a nucleotide exchange factor, facilitates the release of substrate from DnaK by accelerating the release of ADP from DnaK which allows for rapid binding of ATP followed by subsequent release of substrate.

Methodology-

Deletion Mutation Approach

To begin to address the questions raised above and others, we have begun to create a number of different deletion mutants of GrpE that encompass various portions of the different domains and regions of the protein (see Figure 3 and Table 1). Through the creation of various deletion mutant proteins, we hope to gain a better understanding of the role of specific regions of the protein.

Results-

A. Purification of Mutants

Using molecular biology techniques, amplified DNA that codes for various mutant proteins was cloned into a heat inducable over-expression vector (pMLM 158). All of the mutants were expressed and purified from E. coli in the same manner. Figure 4 shows the purification scheme for the GrpE 1-138 deletion mutant protein as an example. Figure 5 shows the purified deletion mutants along with the full-length GrpE which was expressed and purified in a similar manner as the mutant proteins.

B. CD Analysis

Before biochemical analysis can be carried out on the various deletion mutants, protein secondary structural content was probed by circular dichroism spectroscopy. Figure 6 shows the results of the CD analysis of GrpE and the various deletion mutants. All samples of proteins have a concentration of 10 mM except the 1-89 mutant which had a concentration of 10 μM. All have some amount of secondary structure with the 1-89 mutant having the least amount.

C. Chemical Crosslinking with EDC

To begin to understand more about the dimer structure of GrpE, chemical crosslinking (see Figure 7) was used to determine whether a dimer species was forming in solution for the full-length protein and the various deletion mutant proteins. EDC is known as a “zero-length crosslinker” because it will join residues that form a salt bridge within a protein structure. The dimer structure of GrpE was analyzed for potential crosslink sites by looking for salt bridges in the structure (see Figure 8). Table 2 gives the number of potential crosslink sites for full-length GrpE and the various deletion mutants. Figure 9 shows the results of the crosslinking with EDC.

Discussion and Future Work-

A deletion mutant protein predicted to contain only the long α-helical tail region (1-89) does retain some α-helical secondary structure (Figure 6). It cannot, however form a dimer species in solution (Figure 9). The 1-112 mutant is able to form a dimer in solution and interestingly, is the only one that forms higher ordered multimeric structures as well (Figure 9). The 1-138 and 89-197 mutants were also able to form dimers like that of the full-length protein. Thus, any mutant that contained the 4-helix bundle region or part of this region was able to form higher ordered multimeric species in solution.

Future work will involve quantification of the multimeric states of each mutant using gel filtration techniques. Once the specific region for dimer formation is identified individual amino acids will be investigated for roles in dimer formation. Ultimately, we hope to create a mutant of GrpE that will be unable to form a dimer and then test its ability to interact with DnaK, to attempt to answer the question of why GrpE is a dimer.