Mechanistic Analysis of the Hsp70 ATPase Chaperone Cycle

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Members of the heat shock protein 70 (Hsp70) chaperone family are involved in a wide range of cellular processes such as protein folding, translocation of proteins across membranes and the assembly or disassembly of protein complexes as well as degradation of aberrant proteins. Hsp70 chaperones assist protein folding by ATPase-controlled cycles of substrate binding and release. ATP hydrolysis occurs at the highly conserved 45-kDa N-terminal domain, and substrate binding occurs at the 25-kDa C-terminal substrate binding domain. The ATP hydrolysis by Hsp70 is usually slow and brings about conformational change, which enables peptide binding to the C-terminal domain. The ATPase activity of prokaryotic Hsp70,*Escherichia coli* DnaK, is regulated by its co-chaperones DnaJ (Hsp40 homolgue) and GrpE (nucleotide exchange factor). DnaJ binds to the DnaK ATPase domain and accelerates hydrolysis to such an extent that ATP binding by DnaK is the rate-limiting step for hydrolysis. The DnaK-ADP state has a relatively high affinity for the polypeptide substrate. GrpE recognises the DnaK-ADP-substrate complex and promotes ADP/ATP exchange, thereby regenerating the DnaK-ATP state which has a relatively low affinity for the substrate, such that the substrate is released to fold to the native state. The aim of this investigation is to examine the the effects of a His-tag on the ATPase activity of the *E. coli* DnaK. N-terminal His-tagged *E. coli* DnaK was constructed to facilitate its purification, and the ATPase activity of this construct will be compared with that of purified untagged *E. coli* DnaK. Future work will involve a comparative analysis of basal ATPase activity and the effect of Hsp40s (such as DnaJ) and nucleotide exchange factors (such as GrpE) on the ATPase activity of different Hsp70 proteins such as *Agrobacterium tumefaciens* DnaK, parasitic Hsp70s and mammalian Hsp70s.

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