Understanding the allosteric mechanism of the Escherichia coli Hsp70 molecular chaperone, DnaK

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Abstract

The Hsp70 family molecular chaperones prevent protein aggregation under heat shock conditions. They are highly conserved, and have a N-terminal ATPase domain, which binds and hydrolyzes ATP, and a C-terminal substrate-binding domain, which binds unfolded stretches of polypeptides. The communication between the two domains is vital for chaperone function. The nucleotide state regulates the substrate affinity, and substrate binding stimulates the ATP hydrolysis rate. Our study to understand this allosteric mechanism showed that the interdomain linker region is crucial for the stimulation of the ATPase activity. The linker region between the two domains is highly conserved and hydrophobic, and when retained with the ATPase domain (DnaK1–392), poised the domain in the high ATPase activity state (8-fold higher activity). The ATPase domain without the linker residues (DnaK1–388) has ATPase activity comparable to the basal ATPase activity of the intact wild-type protein. When these linker residues are mutated in the ATPase domain construct (DnaK1–392 (L390D/L391D)), the stimulation of the ATPase activity decreases (2-fold higher activity). The same linker mutation in intact DnaK (DnaK1-638 (L390D/L391D)) disrupts the interdomain relationship and the propagation of the allosteric communication in both directions. Therefore, the linker region is the allosteric switch that mediates the communication between the ATPase domain and the substrate-binding domain. In addition to the linker region, there are other structural elements, which are part of the interdomain interface, and we tried to identify these regions by fluorescence experiments. In the ATP-bound state, the two domains are more intimately associated, and the α-helical subdomain of the substrate-binding domain and subdomains IA and IB of the ATPase domain are forming interdomain contacts. In the ADP-bound state, the N-terminus of

the bound substrate peptide is within 30 Å of W102 in the ATPase domain. Several mutations in the substrate-binding domain destabilize the substrate-binding domain and push the conformational equilibrium towards the docked ATP-bound state. This showed that the Hsp70 proteins maintain a delicate equilibrium in order to function. Based on our data, we have proposed a structural model for the allosteric mechanism of DnaK. ^

Subject Area

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