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Dancing Through Life: Allosteric Transitions and Structural Analysis of Hsp70 and Hsp110 Chaperone Proteins

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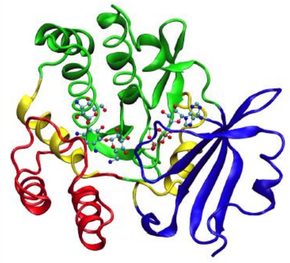

Introduction

The molecular chaperone protein Hsp70 (70 kDa heat-shock protein) is centrally involved in cellular homeostasis by assisting the folding and degradation of protein substrates[10]. Hsp70 is joined by co-chaperones, which contribute to specialized tasks of the Hsp70 complex[7]. Imbalances of this heat shock protein system are believed to be involved with the deregulation of cancer pathways and other human diseases.

Hsp70 allosterically modulates between a closed, ADP bound conformation and an open, ATP bound conformation (see below). Large changes are observed between structures of Hsp70 as ATP binds and allosterically alters protein's shape[6].

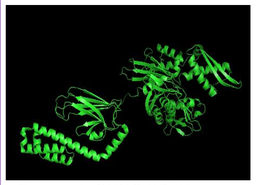

Figure 1. 2KHO XRAY structure of E. coli HSP70 (DNAK)[2]– closed conformation

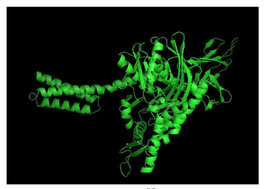

Figure 2. 4JNE Allosteric opening of the polypeptide-binding site when an Hsp70 binds ATP[8].

Hsp70 and Hsp110 are very similar in structure and both consist of a NDB (nucleotide binding domain) a beta-sandwich domain and three alpha helices. When Hsp70 and Hsp110 bind together, this initiates a nucleotide exchange[7].

Objective

Better understanding of how these heat shock proteins work at molecular level will give more clues about biological function. Simulating the formation and function of Hsp70 based chaperone complexes could provide new information about the control and regulation of these processes, as well new areas of exploration for drug discovery.

Experimental Method: Allosteric Explorations

- Relevant protein files, 2KHO[2] and 4JNE[8], were downloaded from the PDB databank and prepped for simulations and analysis.
- CNA (Constraint Network Analysis[5]) was performed to assess thermostability of proteins to identify structural weak spots.
- MDdMD (Maxwell-Demon discrete Molecular Dynamics[9].) was used to create morphed structures of the pathway between Hsp70 open and closed conformations.

Experimental Method (continued)

Protein Docking (Hsp70-Hsp110)

- Relevant protein files (2KHO, 4JNE, 3D2f[7], morphed structures) were renumbered and prepped as needed for docking simulations.
- Ambiguous restraints used for docking were calculated based on previous research[7].
- Uploaded all files to HADDOCK (High - Ambiguity Driven protein-protein DOCKing[3])
- Closed conformation Hsp70 (2KHO) was docked to Hsp110 (3D2F). Process was repeated for docking of open conformation Hsp70 (4JNE) to Hsp110 and for all subsequent morphs of Hsp70 docked to Hsp110.
- PDB files were downloaded from successful simulations and used for data analysis.

Results

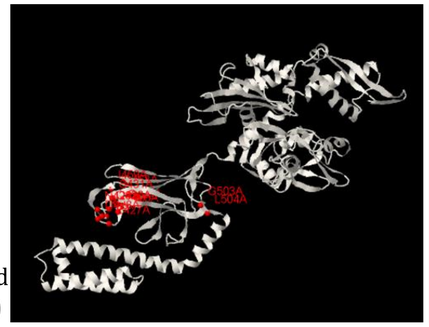

Figure 3 CNA analysis of Hsp70 (2KHO) illustrates weak spots thermally identified on the protein in its open conformation (shown in red).

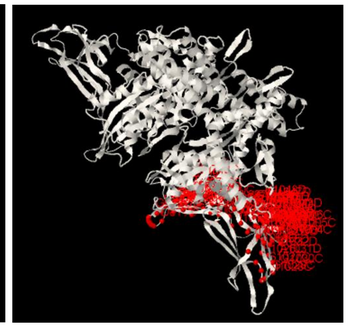

Figure 4 CNA analysis of Hsp70 (4JNE) illustrates weak spots thermally identified on the protein in its closed conformation (shown in red).

Figure 5. Morphed snapshots of Hsp70 (2KHO-4JNE) docked to Hsp110 (3D2F) in HADDOCK simulations. Hsp70 (shown in green) rotates around its flexible linker that connects the NBD (nucleotide binding domain) to SBD (substrate binding domain)(Figure 5). Hsp110 (shown in blue) remains constant throughout simulations. Docked structure of ATP-bound 4JNE to Hsp110 (far right) serves as experimental control. Calculated alignment for experimentally docked ATP-bound Hsp70-Hsp110 complex is within .5RMS of crystal structure or protein complex.

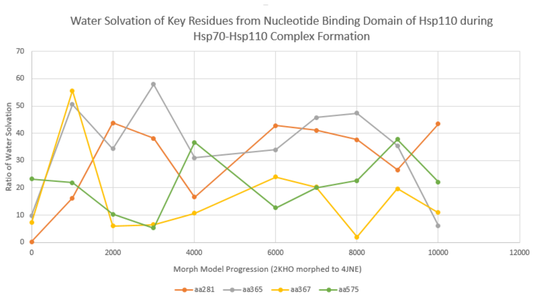

Figure 6. Water solvation of key residues implicated in NBD of complex formation from mutagenesis studies[7]. Water solvation ratio of >50 indicates residue is found on outside of protein complex[4].

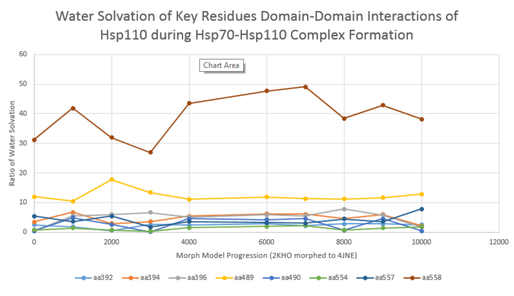

Figure 7. Water solvation of key residues implicated in domain-domain interactions of complex formation from mutagenesis studies[7]. Water solvation ratio of >50 indicates residue is found on outside of protein complex[4].

Conclusions

From the CNA analysis, the weak spots identified in red (Figure 3) correspond the regions of the largest rigid clusters and the domain characterized by beta-pleated sheets. This domain is weak in both the open and closed conformations, indicating that the substrate-binding region is less flexible and more susceptible to degradation as compared to the nucleotide-binding region.

There are many more weak spots identified for the open conformation than closed conformation. This is understandable from a structural level; the ATP-bound complex is transitory and relatively thermodynamically unstable.

Conclusions (continued)

As seen from HADDOCK results (Figure 5) the successful simulation of Hsp70 complexed to Hsp110 in both the open and closed states may combat the criticism that proteins can only be docked in their bound states. From these visualizations, the nucleotide binding domain of Hsp70 is seen to rotate around the flexible linker to join into the plane of the structure of Hsp110 (fully open confirmation). These results also suggest that Hsp110 uses both NBD portion and SBD portion to embrace human Hsp70-NDB counterpart[7].

Results of water solvation analysis[4] show possible transition states and energetics of the complex mechanism. Though further data analysis is needed, the majority of residues feature two peak values of water solvation, which suggests the formation of two transition states involved in the mechanism.

Future Research

Further experimentation will involve isolating morphed snapshots between 2KHO and 4JNE and seeing how these structures can complex to only the NBD of human Hsp70.

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