Matthias P. Mayer

Regulation of Protein Conformation

Introduction

Molecular chaperones of the Hsp70 and Hsp90 families interact with a multitude of proteins in different conformational states. As part of their quality control function they interact with unfolded polypeptides, folding intermediates and misfolded proteins during *de novo* protein folding and refolding of stress-denatured and aggregated proteins. In addition, Hsp70 and Hsp90 proteins cooperatively interact with some 200 native or near-native proteins regulating their activity and stability. Among these proteins are many transcription factors, kinases and other regulatory proteins, which are involved in the control of cell homoeostasis, proliferation, differentiation and programmed cell death. Not surprisingly, Hsp70 and Hsp90 chaperones were recently recognized as potential targets for therapeutic intervention in many pathological conditions, notably cancer, neurodegeneration, and autoimmune diseases.
Our major research goal is to understand the mode of action of Hsp70 and Hsp90 chaperones and how these molecular chaperones influence their substrate proteins. We therefore study the molecular mechanism of Hsp70 and Hsp90, how Hsp70s bind native proteins, how these proteins are transferred with the help of cochaperones onto Hsp90 and how these interactions influence the conformation and activity of these so-called client proteins. We collaborate with Bernd Bukau on some aspects of Hsp70 proteins and chaperone networks.

I. Dynamics and allosteric regulation of Hsp70 proteins

Markus Vogel, Wolfgang Rist, Christian Graf

Crucial to the function of Hsp70 chaperones is the nucleotide-regulated transition between two conformational states, the ATP bound state with high association and dissociation rates for substrates and the ADP bound state with two and three orders of magnitude lower association and dissociation rates. The spontaneous transition between the two states is extremely slow, indicating a high energy barrier for the switch that regulates the transition. We discovered that a universally conserved proline in the ATPase domain constitutes the switch that assumes alternate conformations in response to ATP binding and hydrolysis (Figure 1). The conformation of the proline, acting through an invariant arginine as relay (Arg-relay in Figure 1), determines and stabilizes the opened and closed conformation of the substrate-binding domain and thereby regulates the chaperone activity of Hsp70. In addition, we found that the highly conserved peptide stretch that connects ATPase domain and substrate-binding domain, the so-called linker, mediates the ATP-induced opening of the substrate-binding pocket and the substrate-induced hydrolysis of ATP. In fact, the linker alone is sufficient to stimulate ATP hydrolysis in the absence of a substrate-binding domain. Using amide-hydrogen exchange in combination with mass spectrometry we were able to map ATP-induced conformational changes in both domains.

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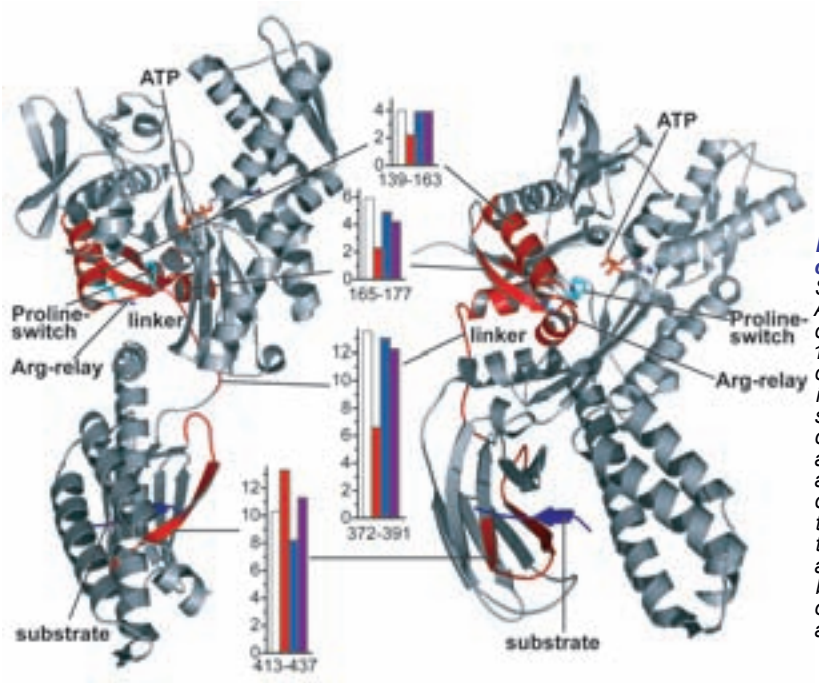

Fig. 1: Nucleotide and substrate-mediated conformational changes is Hsp70 proteins. *Secondary structure representations of ATPase domain (top) and substrate-binding domain (bottom) of Hsp70 (pdb entry code 1YUW and 1DKX; right panel is rotated by ca. 90° as compared to left panel). Colored in red are the segments that change in response to ATP binding and revert partially or completely when ATP and substrate is bound at the same time. Indicated are proline switch, arginine relay, and linker (see text). The diagrams between the structural representations show the deuteron incorporation into the indicated segments of the protein in the absence of nucleotide and substrate (white bars), presence of ATP (red bars), presence of substrate (blue bars), presence of ATP and substrate (purple bars).*

In particular, the linker is completely accessible in the absence of nucleotides and largely protected in the presence of ATP. Conformational changes are also observed in the segment that contains the conserved proline and arginine. We found that substrate binding to the substrate-binding pocket reverts the ATP-induced changes in certain parts of the protein, most notably in the linker and the proline-containing segment thereby pushing the system towards a more ADP-like state. These studies allowed us to map the interdomain signal transduction pathway mediating the mutual allosteric regulation exerted by the two domains onto each other (Figure 1).

II. Conformational changes in Hsp70 substrates

Wolfgang Rist, Fernanda Rodriguez

Using the amide-hydrogen exchange technology we also analyzed two native substrates of the *E. coli* Hsp70 DnaK, the *E. coli* heat shock transcription factor σ^{32} and the F-plasmid replicator protein RepE. In σ^{32} we found that the DnaJ cochaperone binds to the N-terminal domain of σ^{32} and induces an opening of regions adjacent to the DnaK binding site thereby presumably facilitating DnaK binding to σ^{32}. DnaK binding to σ^{32} destabilizes parts of the N-terminal domain. Such a destabilization of σ^{32} by the combined action of DnaJ and DnaK could facilitate inactivation and degradation of σ^{32} *in vivo* and thereby mediate the control of the heat shock response in *E. coli*. In RepE we detected conformational alterations that are dependent on the dimer monomer equilibrium and on DnaK binding to a C-terminal binding site. In both cases, σ^{32} as well as RepE, the interaction with the Hsp70 chaperone has effects on the protein conformation the extent well beyond the area of binding.

III. Conformational dynamics and allostery of Hsp90 proteins

Christian Graf, Marta Stankiewicz

To elucidate the molecular mechanism of Hsp90 proteins we analyzed the conformational dynamics and nucleotide induced conformational changes of Hsp90 proteins from the evolutionary diverse organisms E. coli, yeast and human using amide hydrogen exchange mass spectrometry. Our comparative analysis yielded four novel insights into structure and function of Hsp90 proteins. (1) The conformational dynamics of the eukaryotic Hsp90s is much higher than the dynamics of the prokaryotic Hsp90 homologue HtpG. (2) Nucleotide-induced conformational changes are not restricted to the nucleotide binding site but observed throughout the Hsp90 proteins, most notably in HtpG. (3) ATP-induced conformational changes occur in HtpG in a multistep sequential process, which is rate-limiting for ATP hydrolysis. (4) The two nucleotide-competitive inhibitors tested, geldanamycin and PU-H71, alter the conformational dynamics of Hsp90 in a way that is different from nucleotides and different from each other.

The difference in conformational dynamics of prokaryotic and eukaryotic proteins may have two not mutually exclusive reasons: First, the eukaryotic proteins interact with a large number of native or near-native proteins unrelated in sequence and structure. To bind such a diverse set of clients Hsp90 may have to be very adaptable and high conformational dynamics could guarantee such adaptability. Clients for the prokaryotic HtpG are scarce so far and, in contrast to the eukaryotic Hsp90s, HtpG is not essential for life in most bacteria nor has a deletion of the HtpG encoding gene any detectable phenotype. Second, in contrast to

HtpG, eukaryotic cytosolic Hsp90s are regulated by a large number of cochaperones, which may stabilize Hsp90's conformation according to the needs of the clients.

ATP binding to the Hsp90 proteins induced conformational changes throughout all three investigated Hsp90 proteins but most prominently in HtpG that lead to a more protected tensed state. For the HtpG protein we were able to resolve the transition from the relaxed nucleotide-free state to the tensed ATP-bound state in space and time. We found that ATP binding first induces a small general stabilization in several parts of the protein. Subsequently a succession of more prominent conformational changes occurs starting in the nucleotide binding pocket and propagating towards the N-terminus and the middle domain (Figure 2). Fluorescence measurements supported our findings. A single amino acid replacement variant of HtpG, which has a much lower ATP hydrolysis activity, also undergoes this succession of conformational changes, albeit with slower kinetics. These and other experiments show that the slow stepwise conformational changes are rate-limiting for ATP hydrolysis and therefore act as a timer for the Hsp90 cycle.

Taken together our results suggest that the prokaryotic HtpG is a stand-alone machine, while the eukaryotic cytosolic pendants are highly adaptable machines that are finely tuned by many cochaperones.

IV. Regulation of Hsp70 and Hsp90 by cochaperones: triage decision

To understand how the Hsp70-Hsp90 machinery is regulated by cochaperones we analyze cooperativity and competition between cochaperones. Of specific interest are tetratricopeptide repeats (TPR)-containing cochaperones, which interact with the conserved C-terminal EEVD-motifs of Hsp70s and Hsp90s of the eukaryotic cytosol. Among these TPR cochaperones is the E3 ubiquitin isopeptide ligase CHIP, which links the chaperones to the ubiquitin-proteasomal degradation system and is believed to take part in the triage decision. We asked the question whether Chip preferentially interacts with Hsp70 or with Hsp90 chaperones. In the first case Chip would mainly ubiquitinylate misfolded proteins, while in the second case regulatory proteins would be the main targets for Chip-mediated degradation. We also investigated at which step within the ATPase cycle Chip interacts with the chaperones and whether Chip is able to slow down or accelerate the chaperone cycle.

Using surface plasmon resonance spectroscopy and isothermal titration calorimetry we analyzed the kinetics and thermodynamics of the interaction of Chip with Hsp70 and Hsp90 and compared it to the interaction of the chaperones with other TPR-proteins like Hop and FKBP51/52. We found that the affinity of Chip for Hsp90 is about threefold higher than for Hsp70 but that the affinities and kinetic parameters are very similar to other TPR domain proteins. Therefore the Hsp70-Hsp90 chaperone machinery is a fine tuned system where the TPR cochaperones, including Chip, compete efficiently with each other. We did not find any influence of Chip on the binding of other cochaperones not interacting with the C-terminus or on the ATPase cycle of Hsp70 and Hsp90. Therefore, Chip randomly samples the two chaperones and ubiquitinylates with higher probability those substrates that remain bound to the chaperones for an extentended time period. Such substrates are more likely to be irreversibly misfolded proteins or regulatory proteins in the absence of stimulatory signals. An additional twist in the system comes through the discovery that Chip is subjected to phosphorylation by the MAP-kinase pathway. The functional consequences for the chaperone system are currently under investigation.

Collaborations

We acknowledge our collaborators B. Bukau (ZMBH), C. Georgopoulos (University of Utah, Salt Lake City), F. Hambrecht (IWR Univ. Heidelberg), B. Hu (Keio University, Japan), E. Noessner (LMU Muenchen), S. Rospert (Univ. Freiburg), T. Ruppert (ZMBH), I. Sinning (BZH Univ. Heidelberg), V. Sourjik (ZMBH), W. Voos (Univ. Bonn)

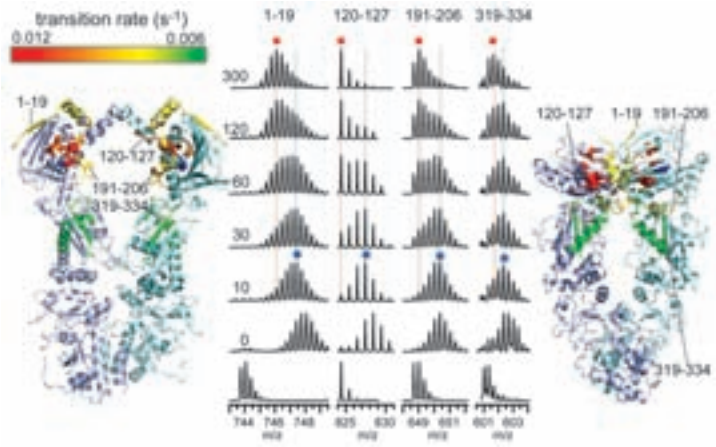

Fig. 2: ATP-induced conformational transition of the E. coli Hsp90 homologue HtpG. *Secondary structure representation of HtpG in the ADP bound state (right panel, PDB entry code 2IOP) and of a homology model of HtpG on the structure of yeast Hsp82 in complex with AMPPNP and the cochaperone Sba1(left panel, PDB entry code 2CG9) colored according to the rate of ATP-induced conversion from the relaxed into the tensed state. Middle panel, mass spectra of selected peptic peptides (amino acid numbers indicated) before incubation in D2O (bottom trace) and after 0, 10, 30, 60, 120, 300 s (from bottom to top) pre-incubation with ATP followed by 10 s pulse-labeling with D2O. The blue circle and the red square indicate the highest peak before and after the slow transition between the relaxed and the tensed state.*

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Honors/Awards

Habilitationspreis der Ernst Schering Research Foundation 2002 (GBM Junior Award)

THESES

Diploma

Stankiewicz, Marta (2006) Production von Hsc70 Substraten in E. coli für Interaktionsstudien.

König, Lars (2007) Membrane Protein Aggregation: Establishment of a model system to study aggregation, disaggregation and degradation of membrane proteins.

Dissertation

Nikolay, R (2006) Investigations of structure, function and regulation of the chaperone-associated ubiquitin E3 protein ligase CHIP.

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Vogel, M. (2007) Investigations on the molecular basis of interdomain communication in Hsp70 chaperones.

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