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The 'active life' of Hsp90 complexes ☆

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ABSTRACT

Hsp90 forms a variety of complexes differing both in clientele and co-chaperones. Central to the role of co-chaperones in the formation of Hsp90 complexes is the delivery of client proteins and the regulation of the ATPase activity of Hsp90. Determining the mechanisms by which co-chaperones regulate Hsp90 is essential in understanding the assembly of these complexes and the activation and maturation of Hsp90's clientele. Mechanistically, co-chaperones alter the kinetics of the ATP-coupled conformational changes of Hsp90. The structural changes leading to the formation of a catalytically active unit involve all regions of the Hsp90 dimer. Their complexity has allowed different orthologues of Hsp90 to evolve kinetically in slightly different ways. The interaction of the cytosolic Hsp90 with a variety of co-chaperones lends itself to a complex set of different regulatory mechanisms that modulate Hsp90's conformation and ATPase activity. It also appears that the conformational switches of Hsp90 are not necessarily coupled under all circumstances. Here, I described different co-chaperone complexes and then discuss in detail the mechanisms and role that specific co-chaperones play in this. I will also discuss emerging evidence that post-translational modifications also affect the ATPase activity of Hsp90, and thus complex formation. Finally, I will present evidence showing how Hsp90's active site, although being highly conserved, can be altered to show resistance to drug binding, but still maintain ATP binding and ATPase activity. Such changes are therefore unlikely to significantly alter Hsp90's interactions with client proteins and co-chaperones. This article is part of a Special Issue entitled: Heat Shock Protein 90 (HSP90).

1. Introduction

Hsp90 is an essential molecular chaperone required for the maintenance, activation or maturation of specific client proteins. Many of these clients are some of the most important proteins involved in signal transduction pathways. They include proteins such as BRaf, ErbB2, Cdk4, and steroid hormone receptors as well as structural proteins such as actin and tubulin (see http://www.picard.ch/Downloads/Hsp90interactors.pdf). The versatility of the Hsp90 system has also enabled viral proteins in many instances to recruit Hsp90 as a chaperone for their own needs [1]. Because Hsp90 is responsible for the activation of such a structurally diverse range of proteins it has evolved to form a variety of complexes each with specific co-chaperones that regulate its ATPase coupled conformational changes.

Understanding the mechanism of ATP hydrolysis is crucial to understanding the role that Hsp90 co-chaperones play and ultimately the mechanism of client protein activation. Direct evidence for this controversial activity [2–4] came from the crystal structure of the N-terminal domain of the *saccharomyces cerevisiae* Hsp90 in complex with ADP, which identified that the essential residues for ATP catalysis were conserved [5]. The focus then moved on to the precise mechanism by which ATP is hydrolyzed [6–9]. Support for the rate-limiting step being structural change [9], rather than ATP hydrolysis [6], emerged from the co-crystal structure between the middle domain of Hsp90 and the N-terminal domain of Aha1 [8]. Aha1 was seen to modulate the so-called catalytic loop of the middle domain of Hsp90 and therefore activate its ATPase activity. Categorical evidence that the formation of catalytically active units of Hsp90 involve a series of complex conformational changes [7] was seen with the full-length structure of Hsp90 in its closed N-terminally dimerized state, stabilized by Sba1 bound between its N-terminal domains [10] (Fig. 1a–b). This revealed the structural changes that form the catalytically active state involve a complicated process of conformational switches in different regions of the protein, which together conspire to assemble the active state [7,9–11]. It is now apparent that all Hsp90 homologs can hydrolyze ATP [12–15]. To understand the role of co-chaperones in Hsp90 complexes it is essential to understand the conformational cycle of Hsp90. Thus, I will give a brief outline of the ATP-coupled structural changes of Hsp90 before discussing the role of co-chaperones in Hsp90.

2. Structural changes and the catalytic unit of Hsp90

To achieve a closed catalytically active state, Hsp90 initially undergoes a structural rearrangement of its ATP lids, represented by residues Gly 94 to Gly 121 (*S. cerevisiae* numbering), which close over the bound ATP. The restructuring of this segment disrupts the packing

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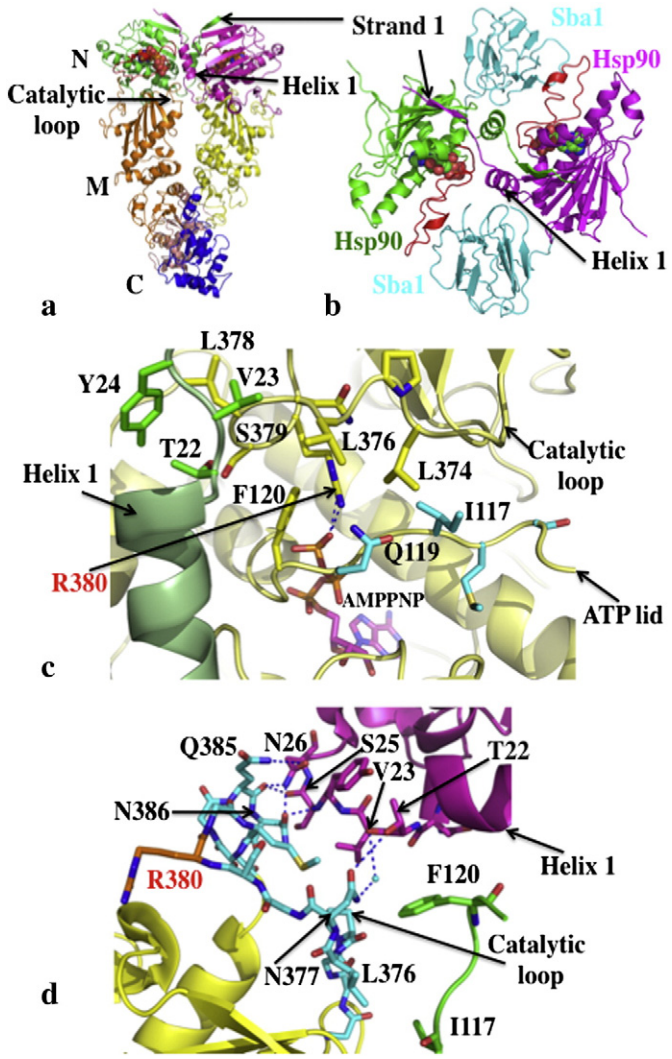

Fig. 1. The catalytically active unit of Hsp90. a), Pymol cartoon of the Hsp90 dimer (pdb, 2CG9; Sba1 not shown). N-terminal domains (N) are in green and magenta, middle domains (M) in gold and yellow and the C-terminal domains (C) in salmon and blue. AMPPNP is shown as spheres bound to the N-terminal domains. b), Stabilization of the N-terminal domains of Hsp90 in their dimerized state by Sba1 (pdb, 2CG9). Sba1 is shown in cyan bound between the dimerised N-terminal domains of Hsp90 (green and magenta). The ATP lids (red) are in their closed conformation. α-helix 1 and β-strand 1, which undergo an exchange of position are indicated. AMPPNP is shown as spheres. c), Stabilization of the catalytic loop (yellow with yellow residues) by interaction between Thr 22 of the neighboring N-terminal domain (pale green with green residues) and Leu 378 of the catalytic loop and between Ile 117 of the ATP lid (yellow with cyan residues) and Leu 374 of the catalytic loop of the same monomer (pdb, 2CG9). Hydrogen bonds between the catalytic Arg 380 and AMPPNP are shown with dotted blue lines. d), Model of the domain interface between the N-terminal- and middle-domain of Hsp90 showing that the catalytic loop may remain in a closed inactive state and is thus not coupled to other structural changes resulting from the closed dimerized state of Hsp90. The middle domain (pdb, 1HK7; yellow) with a closed catalytic loop (cyan, except for Arg 380 which is shown in gold) was superimposed on the full-length closed Hsp90 structure (pdb, 2CG9; N-domain, magenta; middle domain, green). No steric clashes are observed and a number of hydrogen bonds can be formed, which are shown as dotted blue lines. Water molecules are shown as cyan spheres.

of the ATP lid against α-helix 1 of the N-terminal domain. This in turn allows this helix and β-strand 1, residues 1 to 27, of each monomer of Hsp90 to remodel as part of an exchange between the N-terminal domains that occurs during dimerization (Fig. 1a–b). The repositioning of α-helix 1 is important as it sets up the dimerization interface for the N-terminal domains (Fig. 1b). Furthermore, the ATP lid in the closed state allows the catalytic loop residue (Arg 380) of the middle domain access to the ATP-binding site. The ATP lid in the closed position also helps to stabilize the catalytic loop in its open active state, by making a hydrophobic contact between its Ile 117 amino acid residue and Leu 374 of the catalytic loop (Fig. 1c). In addition, the repositioning of α-helix 1 allows its Thr 22 residue to interact with Leu 378 of the catalytic loop of the neighboring Hsp90 monomer and thereby stabilize it in an active state (Fig. 1c).

Recent kinetic analysis [16,17] lends support to the rate-limiting step of ATP turnover being conformational change rather than ATP hydrolysis [6]. The first of these models [17] suggests that following binding of ATP to yeast Hsp90, the conformational changes leading to the catalytically active state involves a transition to two intermediate conformations (Fig. 2). The first, I_1, results from ATP lid closure and release of the N-terminal segment of the N-terminal domain and has the lowest rate constant. I_2 is then formed through dimerization of the N-domains. Finally, a third transition to the catalytically active state occurs by association of the N- and middle-domains and presumably interaction of ATP with the catalytic loop. The ATP is now trapped and committed to hydrolysis. In this model Aha1 bypasses the formation of the I_1 state, thus presumably accelerating the rate-limiting step of the reaction. In another model, binding of ATP to the *Escherichia Coli* Hsp90 homolog, HtpG, leads to a two-phase transition, a rapid change to an intermediate state followed by a slower transition to the T (closed) state (Fig. 2). Interestingly, HtpG lacks an equivalent Aha1 homolog, which might account for this difference, assuming all states have been identified.

The above model suggests that Aha1 modulates specific early conformational switches that accelerate the rate-limiting step of the cycle? However, this might not be as simple as it appears at first sight. The activation of the Hsp90 ATPase activity by full-length Aha1 is significantly stronger than that by the N-terminal domain alone [15]. Thus, it is clear that Aha1 has additional affects on Hsp90 that have not as yet been structurally characterized. However, the N-terminal domain of Aha1 in complex with the middle domain of Hsp90 shows that Aha1 releases the catalytic loop of the middle domain, but this step seems to be, at least in the absence of Aha1, a late step conformational change. Furthermore, release of the catalytic loop might in itself influence earlier steps in the cycle, thus altering the

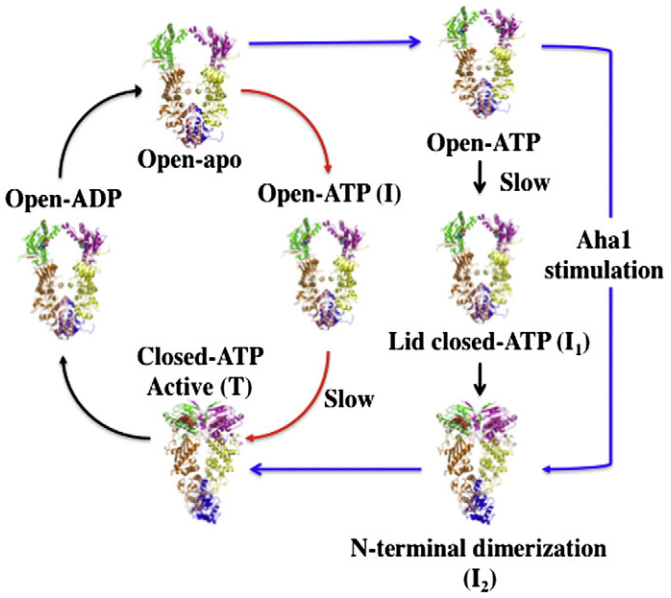

Fig. 2. Kinetic cycles of the *S. cerevisiae* Hsp90 and *E. coli* Htpg. In the yeast cycle (blue and black arrows) the conformational changes leading to the catalytically active state (Closed-ATP-Active) involves transition via two intermediate conformations (I_1 and I_2). Aha1 is supposed to accelerate the cycle by bypassing the I_1 sate. In the *E. coli* cycle (red and black arrows) a two-phase transition via an intermediate state (I) leads to the closed active (T) state. The slowest step in the cycles, both representing conformational change, are indicated. The open, closed and active state as well as the nucleotide state of the chaperone throughout the cycle is indicated.