Interaction of *E. coli* Hsp90 with DnaK Involves the DnaJ Binding Region of DnaK

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Abstract

The 90-kDa heat shock protein (Hsp90) is a widely conserved and ubiquitous molecular chaperone that participates in ATP-dependent protein remodeling in both eukaryotes and prokaryotes. It functions in conjunction with Hsp70 and the Hsp70 cochaperones, an Hsp40 (J-protein) and a nucleotide exchange factor. In *Escherichia coli*, the functional collaboration between $Hsp90_{Ec}$ and Hsp70, DnaK, requires that the two chaperones directly interact. We used molecular docking to model the interaction of $Hsp90_{Ec}$ and DnaK. The top-ranked docked model predicted that a region in the nucleotide-binding domain (NBD) of DnaK interacted with a region in the middle domain of $Hsp90_{Ec}$. We then made substitution mutants in DnaK residues suggested by the model to interact with $Hsp90_{Ec}$. Of the 12 mutants tested, 11 were defective or partially defective in their ability to interact with $Hsp90_{Ec}$ *in vivo* in a bacterial two-hybrid assay and *in vitro* in a bio-layer interferometry assay. These DnaK mutants were also defective in their ability to function collaboratively in protein remodeling with $Hsp90_{Ec}$ but retained the ability to act with DnaK cochaperones. Taken together, these results suggest that a specific region in the NBD of DnaK is involved in the interaction with $Hsp90_{Ec}$, and this interaction is functionally important. Moreover, the region of DnaK that we found to be necessary for $Hsp90_{Ec}$ binding includes residues that are also involved in J-protein binding, suggesting a functional interplay among DnaK, DnaK cochaperones, and $Hsp90_{Ec}$.

Introduction

The 90-kDa heat shock protein (Hsp90) is a highly ubiquitous and evolutionarily conserved molecular chaperone [1–5]. It is essential in eukaryotes, where it is involved in the folding, stability, and activation of more than 200 client proteins including many transcription factors, steroid hormone receptors, and protein kinases [4,6,7]. In addition, Hsp90 stabilizes and activates oncoproteins and therefore is a potential target for drug discovery for the treatment of cancer [8]. *Escherichia coli* Hsp90, referred to as $Hsp90_{Ec}$ and encoded by *htpG,* is an abundant protein and is further induced upon heat shock and other stress conditions [9]. Strains lacking $Hsp90_{Ec}$ exhibit modest phenotypes, including slow growth at elevated temperature [9], accumulation of aggregated proteins at high temperature [10], loss of adaptive immunity conferred by the CRISPR system [11], decreased ability to form biofilms at elevated temperature [12], and decreased ability to swarm [13]. Overexpression of $Hsp90_{Ec}$ causes defects in cell division that results in filamentous cells and SDS sensitivity [14].

Hsp90 is a homodimer with each monomer consisting of three domains: an N-terminal domain that possesses an ATP binding site [15,16], a middle domain containing residues that participate in binding client proteins [7,14], and a C-terminal domain that is essential for dimerization and is also involved in client binding [3,14]. ATP binding and hydrolysis by Hsp90 trigger large conformational changes that are necessary for the cycle of client binding, remodeling, and release [4,15–21]. The Hsp90 dimer exists in a predominantly open V-shaped conformation in the absence of nucleotide with the protomers interacting via the C-terminal domain [21]. ATP binding triggers the closing of the ATP lid on the ATP-binding site, followed by the dimerization of the two N-terminal

domains [4,18,21,22]. ATP hydrolysis and ADP release lead to the dissociation of the N-domains [4,18,21], and Hsp90 returns to the open conformation [18–20,22]. Cochaperones and client protein binding bias the Hsp90 chaperone cycle and stabilize or destabilize various conformations of Hsp90 [1,3,4].

Hsp90 functions with the Hsp70 chaperone system in protein activation and remodeling [1,5,23]. Eukaryotic Hsp70 and its prokaryotic homolog, DnaK, are highly conserved proteins [24–27]. Hsp70/DnaK is composed of an N-terminal nucleotide-binding domain (NBD) and a C-terminal substrate-binding domain (SBD) that are connected by a flexible linker [25]. It collaborates with two cochaperones, an Hsp40 (J-domain protein) and a nucleotide exchange factor (NEF). The Hsp40 protein stimulates ATP hydrolysis by Hsp70/DnaK and presents the substrate to Hsp70/DnaK, while the NEF stimulates nucleotide exchange by Hsp70/DnaK [24,27,28].

In addition to collaborating with the Hsp70 chaperone system, eukaryotic Hsp90 also functions with numerous cochaperones, including Hop/Sti1, Aha1/Hch1, p23/Sba1, Cdc37, and Sgt1 [1,15,16]. Cochaperones regulate Hsp90 in various ways, such as imparting client protein specificity, modulating ATPase activity, or stabilizing specific Hsp90 conformations [1,4]. Moreover, Hop/Sti1 stabilizes the interaction between eukaryotic Hsp90 and Hsp70 by simultaneously interacting with tetratricopeptide repeat domains at the extreme C terminus of each chaperone. In contrast to eukaryotes, bacterial Hsp90 functions with the DnaK chaperone system independently of other Hsp90 cochaperones.

Protein reactivation by bacterial Hsp90 *in vitro* is simpler in its requirements than its eukaryotic homolog, requiring only DnaK and a J-domain protein [23,29]. GrpE, the bacterial NEF, stimulates reactivation but is not essential [23]. ATP hydrolysis and client binding by both chaperones are essential for reactivation [30]. Moreover, bacterial Hsp90 physically interacts with DnaK *in vivo* and *in vitro* [29,30] through a region identified on the M-domain of $Hsp90_{Ec}$ [30].

In the work presented here, we examined the collaboration between $Hsp90_{Ec}$ and DnaK. We used molecular modeling to identify a region on DnaK that interacts with the middle domain of $Hsp90_{Ec}$. We then constructed DnaK mutants in some of the residues suggested by the docked model and tested the mutants for defects in interaction and functional collaboration with $Hsp90_{Ec}$. We found that the region of DnaK involved in the physical and functional interaction with $Hsp90_{Ec}$ comprises residues in the DnaK NBD. This region of DnaK overlaps the region where DnaJ binds, suggesting a mechanism where $Hsp90_{Ec}$ directly interacts with a client-bound DnaK–DnaJ complex to displace DnaJ and promote the transfer of substrate to $Hsp90_{Ec}$.

Results

Identification of residues in DnaK potentially involved in protein interactions with $Hsp90_{Ec}$

We previously showed that *E. coli* DnaK collaborates with $Hsp90_{Ec}$ in protein reactivation and that the two chaperones physically interact through a region in the middle domain of $Hsp90_{Ec}$ [23,30]. To elucidate the region of DnaK essential for binding $Hsp90_{Ec}$, we used molecular docking to predict the potential interactions between the two chaperones. Four combinations of $Hsp90_{Ec}$ and DnaK molecules available in the protein data bank were tested [21,31,32]: (1) ADP-bound DnaK with apo $Hsp90_{Ec}$, (2) ADP-bound DnaK with ADP-bound $Hsp90_{Ec}$, (3) ATP-bound DnaK with apo $Hsp90_{Ec}$, and (4) ATP-bound DnaK with ADP-bound $Hsp90_{Ec}$ (see Materials and Methods). Without imposing restraints, the proteins were docked using ZDOCK [33,34], and the top model for each combination was selected based on the lowest energy using ZRANK [35]. Three of the four models (2, 3, and 4 above) could be eliminated since they predicted interacting regions that were inconsistent with earlier work [30] or with results shown in Supplementary Information (Supplementary Results and Supplementary Figs. S1–S5 and Supplementary Tables S2–S4).

The model of the complex of ADP-bound DnaK and apo $Hsp90_{Ec}$ predicted that DnaK interacted exclusively with residues on the $Hsp90_{Ec}$ middle domain, many of which had been shown experimentally to be involved in binding DnaK [30] (Fig. 1a and Table 1). To gain additional evidence for this model, we constructed two $Hsp90_{Ec}$ mutants with substitutions in residues

Fig. 1. Regions of interaction on DnaK and $Hsp90_{Ec}$. (a) Docked model of the apo structure of $Hsp90_{Ec}$ [21] and ADP-bound DnaK [31] as determined using ZDOCK and ZRANK and described in Materials and Methods. $Hsp90_{Ec}$ is shown as a surface rendering with one protomer in dark gray and one protomer in light cyan. The DnaK interacting region of $Hsp90_{Ec}$ [30] is shown in red, while the client binding region is in blue [14]. DnaK in the ADP-bound conformation is shown as a ribbon model with the NBD in light orange and the SBD in light gray. (b) DnaK in the ADP-bound conformation [31] showing residues (purple) on DnaK within 8 Å of $Hsp90_{Ec}$ as predicted by the docked model in (a). In (b–d), DnaK is shown as a surface rendering with the NBD in light orange and the SBD in light gray. (c) DnaK in the ADP-bound conformation [31] showing residues (green) experimentally identified as interacting with DnaJ [38–40]. (d) DnaK in the ATP-bound conformation [32] showing residues (purple) on DnaK within 8 Å of $Hsp90_{Ec}$ as predicted by the docked model in (a). In the ATP-bound conformation, only some of the DnaK residues within 8 Å of $Hsp90_{Ec}$ in the model are surface exposed. Images in (a–d) were made using PyMOL (Schrodinger, LLC; www.pymol.org).