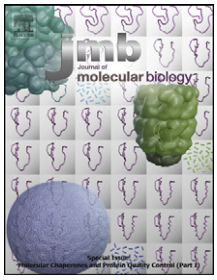

How Hsp70 Molecular Machines Interact with Their Substrates to Mediate Diverse Physiological Functions

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Abstract

Hsp70 molecular chaperones are implicated in a wide variety of cellular processes, including protein biogenesis, protection of the proteome from stress, recovery of proteins from aggregates, facilitation of protein translocation across membranes, and more specialized roles such as disassembly of particular protein complexes. It is a fascinating question to ask how the mechanism of these deceptively simple molecular machines is matched to their roles in these wide-ranging processes. The key is a combination of the nature of the recognition and binding of Hsp70 substrates and the impact of Hsp70 action on their substrates. In many cases, the binding, which relies on interaction with an extended, accessible short hydrophobic sequence, favors more unfolded states of client proteins. The ATP-mediated dissociation of the substrate thus releases it in a relatively less folded state for downstream folding, membrane translocation, or hand-off to another chaperone. There are cases, such as regulation of the heat shock response or disassembly of clathrin coats, however, where binding of a short hydrophobic sequence selects conformational states of clients to favor their productive participation in a subsequent step. This Perspective discusses current understanding of how Hsp70 molecular chaperones recognize and act on their substrates and the relationships between these fundamental processes and the functional roles played by these molecular machines.

Introduction

Hsp70 molecular chaperones have a deceptively simple mechanism of action: they bind via their substrate-binding domain (SBD) to short polypeptide sequences with recognition motifs that are not stringently defined but can be identified by common features. The affinity of Hsp70s for their substrates is modulated allosterically by nucleotide binding to their N-terminal actin-like nucleotide-binding domain (NBD), with ATP binding causing a reduction in affinity and ADP binding leading to high substrate affinity. The past few years have provided deep insights into the structural basis of Hsp70 allostery, much of it was based on the *Escherichia coli* Hsp70 family member DnaK [1,2]. In the ADP-bound state of DnaK, the NBD and the SBD are largely independent, behaving similar to the separate domains connected by a flexible linker [3]. Upon ATP binding, the two domains are intimately docked with a conserved hydrophobic sequence in the interdomain linker forming a key part of the packing interface between domains [4–6]. Substrate binding to the ADP-bound DnaK is characterized by slow on–off kinetics. In the presence of ATP, substrate binding stimulates the ATP hydrolysis rate of the NBD, and the on and off rates for substrate binding are accelerated, leading to lower overall affinity and consequently favoring substrate release [7]. Atomic structures are now available for both ADP-bound [3] and ATP-bound [4,5] DnaK, and an activated state of DnaK with both ATP and a substrate peptide bound was recently described, in which the hydrophobic linker sequence is associated with the NBD but the

domains are not fully docked on each other [8]. In physiological contexts, the allosteric cycle of Hsp70s is modulated by co-chaperones including nucleotide exchange factors, which stimulate exchange of ADP for ATP, as well as J-proteins, which both facilitate substrate delivery to Hsp70s and catalyze ATP hydrolysis [2]. The impact of J-proteins in modulating Hsp70-substrate interactions is of great importance to the interplay of this chaperone system with its clients and will be discussed in greater detail in a later section. While the allosteric mechanism of Hsp70s has been elucidated to a great extent, our understanding of the impact of Hsp70 chaperone actions on their substrates remains shallow despite the fact that this is the essence of Hsp70 physiological functions.

Hsp70s facilitate a stunning array of diverse functions. Their ability to do so is a consequence of their intrinsic allosteric mechanism coupled with their ability to cooperate with upstream and downstream chaperone partners. For example, all Hsp70s partner with J-protein family members, which are highly diverse, specialized, and spatially restricted [9]. Hsp70 actions include optimization of folding and minimization of aggregation, both early in the biogenesis of proteins and upon stress-induced unfolding; shepherding proteins across membranes in translocation processes that require substrates to remain unfolded; promoting disassembly of large protein complexes; working with partner chaperones to mediate disaggregation reactions; and hand-off of substrates to downstream chaperones in folding and assembly reactions. When proteins fail to acquire the proper structure for downstream steps, Hsp70s work with their co-chaperones and partner chaperones to provide a quality control mechanism that includes shunting non-competent proteins to degradation [10,11].

Despite this physiological diversity, all Hsp70 functions share a reliance on their ability to bind and release short unstructured sequences in their substrates. Importantly, the extent to which this binding reaction remodels the substrate and alters its conformational ensemble is critical to the biological outcome. Questions that must be addressed to get to the heart of the action of Hsp70s on their substrates include the following: what is the nature of the recognition of substrates (including sequence and conformational requirements in the substrate for binding), whether and how the binding and release by the chaperone remodel the substrate, whether there is a direct mechanical action of Hsp70s on substrates, how/whether subsequent processes involving the substrate (folding, translocation, etc.) are favored because of the action of the Hsp70, and how hand-off to downstream chaperones is facilitated by Hsp70 action.

In this Perspective, we discuss what is known about the answers to these questions. This brief review makes it clear that current knowledge is limited. With a few notable exceptions, we lack adequate fundamental understanding of the nature of Hsp70 chaperone/substrate interactions to elucidate the related physiological functions. The reasons for this are not unusual: most of our current understanding is based on model systems with purified components, in isolation from the full system responsible for the physiological function. In particular, in the case of Hsp70s, the lion's share on our understanding of how they interact with substrates derives from studies of short peptides as models for protein substrates. Moreover, most experiments have been carried out on purified Hsp70s in isolation from partner chaperones. Thus, there is a pressing need to take our knowledge of Hsp70-substrate interactions to the next level of complexity. Here, we discuss examples of published work related to Hsp70/substrate interactions, and we apologize in advance to those whose work we do not cite because of the limitations of length and scope of this Perspective.

The Basis of Substrate Recognition by Hsp70s

Structural descriptions of substrate binding: Peptides

It was discovered early on that Hsp70s bind a variety of peptides 7 residues in length and that these fragments, like protein substrates, activate the ATPase activity of the chaperone [12–14]. From that point on, there has been heavy reliance on peptide models to understand the basis of substrate recognition by Hsp70s. Peptide library and phage display studies with several Hsp70s showed a preference for hydrophobic residues and positive charge, but without any clear binding motif [14–18]. Nonetheless, enough sequence bias has been extracted from these studies to develop predictive algorithms to identify potential Hsp70 binding sites [14–19]. From the earliest phage display work, it appeared that sequence preferences may differ from one Hsp70 to another [14,16], but the overlap in binding capacity is high and it remains an open question to what extent Hsp70s have binding sequence biases [20]. Current thinking is that the J-protein partners of Hsp70s provide most of the physiological substrate specificity [9,21], which is also consistent with the greater number of different J-proteins than Hsp70s in a given cellular compartment and particularly the larger number in eukaryotes relative to prokaryotes.

The first atomic-level structural description of substrate binding by an Hsp70 revealed that the 7-residue model peptide NRLLLTG (called “NR”) bound in an extended, polyproline-like conformation