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Molecular Chaperones: Heat Shock Protein 70

by

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Abbreviations:

BiP: immunoglobulin heavy chain binding protein
Hsf-1: heat shock factor 1
MHC: major histocompatible complex
HPD motif: tripeptide H33, P34, D35.
Hsp: heat shock protein

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Abstract

Heat shock proteins (Hsp) are a family of proteins that primarily function as molecular chaperones. Heat shock proteins can be classified in 5 groups based on molecular weight: 70 kDa, 90 kDa, 50-60 kDa, 20-30 kDa, and 100-110 kDa. Nevertheless, much of the work on Hsps has focused on Hsp70. Some Hsp70s are constitutively expressed (Hsc70); some can be induced during stress. The functions of Hsp70 and Hsc70 have some similarities and interact one another. Although the exact functions of Hsp70 are not fully understood, it is known to be central components of the cellular chaperone system that assists folding and degradation of proteins. The structure of Hsp70, the function cycle of molecular chaperone, and the mechanism of induction of Hsp70 will be discussed below.

Introduction

The induction of heat shock protein was originally observed in *Drosophila* exposed to heat shock [1]. Hsps are abundant proteins in cells. Up to 5% of the cellular proteins are Hsps [2]. It is almost a universal phenomenon that cells induce Hsp or related proteins in response to a variety of environmental stresses or unfavorable conditions. Hsp are molecular chaperones, they help fold newly synthesized proteins and also assist in the refolding of damaged proteins or facilitate the degradation of the severe damaged proteins. Their activity requires ATP and cooperation with cofactors that control the Hsp70 ATPase cycle and target the chaperone to specific substrates. The energy of ATP is used to refold proteins. Interestingly, Hsps are not only induced by elevated temperature, they are also induced by amino acid analogue [3], transition metals [4], and oxidative stress [5].

Structure

Heat shock proteins are highly conserved among all species, and so far, there are no known organisms or cell types without heat shock proteins. Virtually all Hsps have an ATP binding sequence at the amino-terminal end, and a substrate binding sequence at the C-terminal. The sequence of ATP binding is highly conserved across species [6], however the substrate binding sequence is less conserved. The GroE protein family is Hsp homologue machinery in bacteria, DanK is homologue in *E. coli*, and BiP is protein in endoplasmic reticulum. Up to now there is no known complete structure of a full length Hsp70 protein.

The structure of ATPase domains of Hsc70, Hsp70, and DanK are very similar to the protein fold of actin [7]. They comprise of two globular subdomains, separated by a deep central cleft and connected by two crossed a-helices. ATP, one Mg^{2+}, and two K^{+} ions are bound at the

bottom of the cleft by interactions with both subdomains and connecting helices [8]. Figure 1 shows both the structures of the ATPase and substrate binding domain.

The substrate binding domain represents a novel protein fold that consists of a ß sandwich arranged in two sheets with four antiparallel ß stands in each. The ß sheets form the bottom of substrate binding activity. The substrate is buried in the loops which connect the ß sheets. These connecting loops are stabilized by a long a helix (Figure 1B). This loop functions as a lid that opens or closes the substrate binding site in an ATP dependent manner [9].

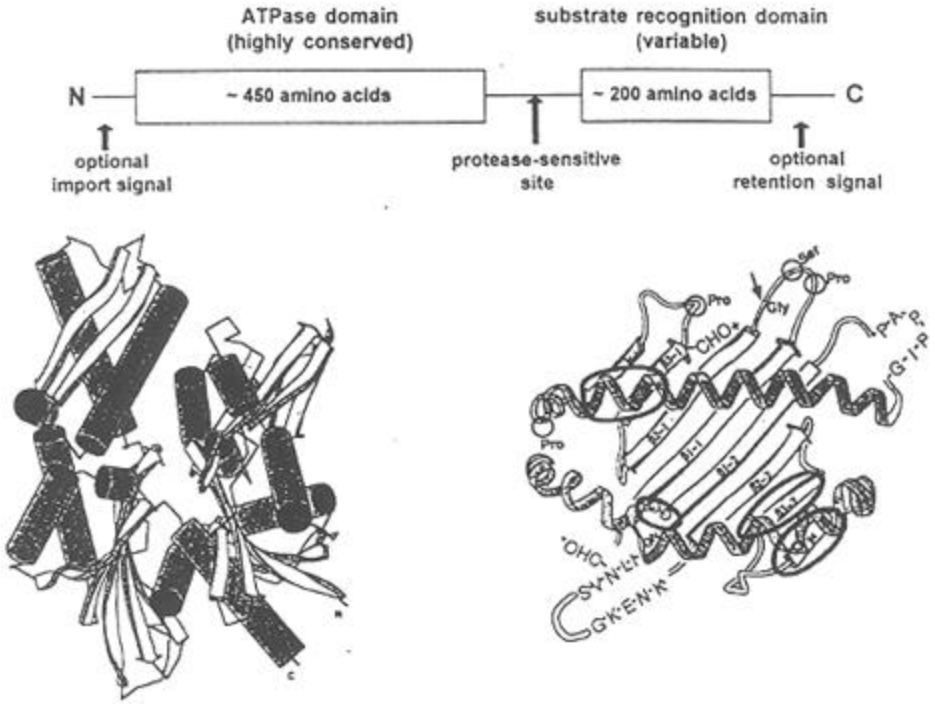

Figure 1: Structure of Hsp70: the ATPase domain and substrate binding domain.
Some Hsp70 contain N-terminal extensions for targeting to the endoplasmatic reticulum or to mitochondria. Bip contains a C-terminal extension involved in retention of the protein in the ER. The structure of ATPase domain (A) was derived from bovine Hsc70. The substrate binding domain (B) was modeled on the structure of human MHC class I molecules. Adapted from [8].

When Hsps function as the molecular chaperone, they must work with a cofactor. The cofactor is Hdj-1 in human, and DnaJ in *E. coli* [8]. DnaJ proteins are multidomain proteins which share a highly conserved signature motif of approximately 78 amino acids, the J-domain. They frequently contain a glycine/phenylalanine rich region, a Zn-finger domain and a C-

terminal domain. The J domain is comprised of four helices (shown in Figure 2). The antiparallal helices 2 and 3 form a coiled coil, which are connected by a flexible turn containing the highly conserved HPD motif. Helices 1 and 4 are perpendicular to helices 2 and 3. The J domain interacts with its Hsp partner to control their function cycle.

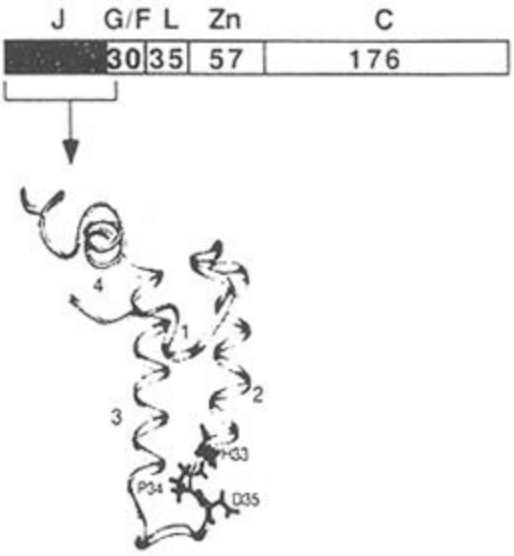

Figure 2: Structure of DnaJ. Linear representation of *E.Coli* DnaJ. J-domain; G/F, region rich in glycine/phenylalanine; L, linker, Zn, Zn-finger motif, C, C-terminal domain. The number of amino acids in each domain is given in the scheme. Under the diagram is the NMR structure of the J-domain. Adapted from [8].

The function cycle of chaperone

All molecular chaperones recognize hydrophobic features of unfolded substrate polypeptides, thereby prevent from binding with folded proteins. Hsp70 uses ATP to control binding and dissociation of substrate polypeptides. The function cycle of DnaK system is well studied. Here we will discuss the function cycle using DnaK chaperone system.

The ATPase cycle of DnaK is an alteration between two conformational states, the ATP state with low affinity for substrates (substrate binding pocket opened), and the ADP state with high affinity for substrates (substrate binding pocket closed) [2].

In the kinetics of these two conformational alterations, DnaJ and GrpE (nucleotide exchange factor) play critical roles. In the ADP bound form, DnaK can rapidly bind to substrates, but the chaperon-substrate complex dissociates very fast, effectively. Prevention of aggregation of misfolded protein substrates is impossible in such a short life of chaperone-substrate complex. In the ADP bound form, DnaK is too slow in protein binding to prevent protein aggregation. To solve this dilemma, DnaK must bind to substrate effectively in ATP bound form; subsequently hydrolyze ATP to stabilize the substrate-chaperon complex. However, the intrinsic ATPase activity of DnaK is very low (approximately 0.04 min^{-1}). The DnaK cofactor can accelerate the ATP hydrolysis dramatically with increasing the rate ?-phosphate cleavage, which is rate-limiting step in this process. The ?-phosphate cleavage can be increased over 1600-fold by saturation concentration of DnaJ [10].

ADP release is faster than ATP hydrolysis, but is still very slow. GrpE increases ADP dissociation by 5000-fold, thereby allowing ATP to rebind to the DnaK with fast kinetic (k_{on} = 90 min^{-1}) [8].

The function cycle involves two crucial processes: one is substrate binding, the other is ATP hydrolysis. It is unclear that which process is the initial event. One model proposed is that the cycle starts with the transition association of substrate peptide to the DnaJ domain (Figure 3). Then this complex binds to ATP DnaK complex with transferring the substrate to the DnaK binding pocket. At the same time the ATPase activity is stimulated by DnaJ, which hydrolyzes ATP to ADP and release of the DnaJ domain. Binding to GrpE helps DnaK release ADP, allowing DnaK bind to ATP, thus converting DnaK to the low affinity form for substrates. This released substrate may become folded or ready to enter a new cycle binding or release to DnaK

[7]. The data available does not exclude the possibility that the cycle starts with association of ATP and DnaK.

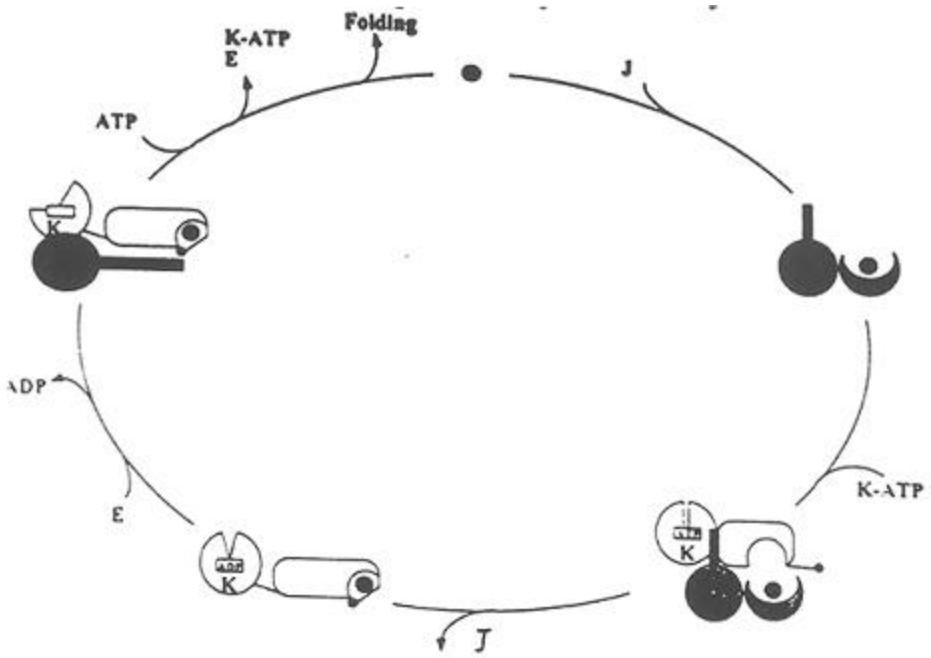

Figure 3: Model for function cycle of DnaK chaperone system. Adapted from [8]
The substrate binds first to the DnaJ, which subsequently recruits DnaK-ATP. The substrate is shuttled into the binding pocket of DnaK and the J-domain of DnaJ stimulates DnaK's ATPase activity simultaneously. The substrate is now stably bound to DnaK-ADP and DnaJ may leave the complex. GrpE interacts with the DnaK-ADP-substrate complex and induces the ADP release. ATP binds rapidly to DnaK, thus reducing DnaK's affinity to the substrate. The substrate is released and partitions between folding or rebinding to DnaJ.

Heat shock response

The heat shock protein 70s have a variety of functions, such as translation, protein translocation, proteolysis and protein folding or chaperoning, suppressing aggregation and reactivating denatured proteins [10]. The thermotolerance of cell or tissues after a heat stress is mostly dependent on the induction of Hsp70.

The synthesis of Hsp70 is mediated by the transcription factor-heat shock factor 1 (Hsf-1). In unstressed condition, monomeric forms of Hsf-1 are maintained as non-DNA binding complex. Negative regulation is relieved by the accumulation of stress generated denatured protein that is

prone to form aggregates. Hsf-1 then undergoes trimerzation, and binds to DNA to activate transcription of Hsp70. This process is triggered by the presence of denatured protein. Conditions that stabilize proteins can inhibit the heat shock response [8].

Though the denatured protein is identified as an initial signal for induction of Hsp70, the question is remained to be answered whether any denatured proteins or some specific denatured proteins are the exact initial signal. Lepock *et al.* found that some thermolabile proteins, at physiological temperature, the native folded conformations are only slightly more stable than their unfolded conformations. As the temperature increases, they are easy to convert to unfolded forms [11]. They also found that these denatured, unfolded proteins were not random coils, instead, they exhibit characteristics of a molten globule, which has a hydrophobic core and residue secondary features [11].

Hsp70 and oxidative stress

As we know, oxidative stress is one of the inducers of Hsp70. Superoxide generator menadione is a very good inducer of Hsp70 [12]. Surprisingly, ionizing radiation administered under oxygenated conditions was a very poor inducer of heat shock response [13]. The distribution of reactive oxygen species produced by a photon is significantly different from those produced by metal-catalyzed oxidation system. The former causes protein fragmentation, while the latter causes protein aggregation which is the trigger of heat shock response [14].

Oxidative stress resulting in non-native modification of protein thiols can cause thermal stable proteins to become destabilized and undergo thermal denaturation at physiological temperature. These thiol modified proteins unfold and form a similar structure as produced by a thermal stress, a molten globule intermediate, which is prone to aggregate.

Summary

Hsp70 are a family of proteins, which can be expressed constitutively or induced by stresses. They have many functions in thermotolerance and immune response. Molecular chaperone is one of the most important functions. The function cycle of molecular chaperone is the alteration of two conformations: the ATP bound low substrate affinity state, and the ADP bound high substrate affinity state. Hsp synthesis is dependent on the activation of Hsf-1. Activation of Hsf-1 is triggered by protein aggregation, which can be caused by a variety of stresses, such as heat shock, transition metals, and oxidative stress.

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