Original research / Orijinal araştırma

Is Hsp70 a folder or a modulator protein?

Hsp70 katlayıcı mı yoksa modulatör protein mi?

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Abstract

Proteins are important macromolecules for cellular activity; intact and correctly folded proteins are required for normal cellular functions. Therefore, a cell has a unique machinery, using heat shock proteins (Hsp), to fold other proteins in their correct three dimensional forms. Several stress factors may cause partial or total destruction of native protein structures. This destruction can be reversible for some of the proteins. Heat shock proteins help some of the unfolded proteins to refold their native states and direct irreversibly denatured proteins to lysosomes for degradation. Hsp70 is at the center of this unique mechanism. Hsp70s also harbor other biochemical functions such as ATPase activity. This article discussed some of the functions of yeast Hsp70.

Keywords: Heat shock proteins, cellular function

Özet

Proteinler hücresel aktivite için önemli makromoleküllerdir, normal hücresel fonksiyonlar için bozulmamış ve doğru katlanmış proteinler gerekir. Dolayısı ile bir hücre proteinlerini doğru üç boyutlu halde tutabilmek için, ısı şok proteinlerini kullanan oldukça özgün bir mekanizmaya sahiptir. Birçok stres faktörü protein yapılarını kısmen veya tamamen bozabilir. Bu bozuklukların bazıları giderilebilirken bazılarını onarmak mümkün olmaz. Isı şok proteinleri bir yandan katlanmamış proteinlerin doğal yapılarını kazanmalarına yardım ederken bir yandan da denatüre proteinleri parçalanmaları için lizozoma yönlendirir. Hsp70'ler aynı zamanda ATPaz gibi aktivitelere de sahiptirler. Bu yazıda maya Hsp70'in bazı fonksiyonları tartışıldı.

Anahtar sözcükler: Isı şok proteinleri, hücresel işlev

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Introduction

Heat shock protein 70 (Hsp70) has a unique structure for folding of the substrate proteins (1-3). Misfolded proteins form aggregates that cause many neurodegenerative disorders including Alzheimer's, Parkinson's, Huntington's, Creutzfeldt-Jakob, cystic fibrosis and Gaucher's diseases (2-4). Hsp70 with its co-chaperones and co-operating chaperones constitute different chaperone folding networks (4-7). Each of these networks serves for a different biochemical function that has to be elucidated. Hsp70 has several different isoforms in a cell. More than one form can be found even in the same compartment of a cell. Yeast cytosol has two different Hsp70 proteins, Ssa and Ssb. Ssa has four, and Ssb has two different forms. Besides, Hsp70 also has a cognate form which is expressed constitutively (8, 9). The reasons for why a cell requires several redundant Hsp70 isoforms have not as yet been clarified.

Potential use of biochemical properties of heat shock proteins has been investigated by many research groups, working in biotechnology and pharmaceutical areas. For example, inclusion bodies during recombinant enzyme production may be co-expressed with heat shock proteins. Hsp70 consists of three domains: N-terminal ATPase domain, substrate-binding domain, and C-terminal domain. ATP hydrolysis by the ATPase domain produces energy, which is transmitted to the substrate binding domain. The latter domain has a hydrophobic cavity and a cover lid on the top. Alternating opening and closing of the lid is coordinated by ATP hydrolysis. Thus, ADP-bound state of Hsp70 is more stable than that of ATP. The cavity provides a hydrophobic environment, helping unfolded proteins to fold to their native state.

Hsp70 acts together with a number of coordinating proteins such as nucleotide exchange factor, Hsp90, Hsp100 and several co-chaperones. Nucleotide exchange factor removes ADP from Hsp70 and enables another folding cycle to start (10, 11). Substrate protein aggregates form a huge chunk on which only Hsp104 can act. Hsp100 also contains ATP and peptide binding domains. The protein forms six-membered ring complexes. Two rings form a dimer by interacting with each other at their O-ring surfaces. Hsp100 can solubilize aggregated proteins as opposed to other chaperones in the family. Hsp100 has a deep hole in the middle of its dimeric structure. Hsp100 process the substrate by passing it from this hole probably to untangle it. It chops off the aggregate and direct the unfolded substrate protein to Hsp70. Thus, Hsp70 and Hsp40 coordinately process the substrate proteins (12-16). All of the members of Hsp40s have a J domain, interacting with the Hsp70 ATPase domain. This interaction stimulates the ATPase activity of Hsp70. Thus, the Hsp70-Hsp40 pair either fold substrate proteins or transport them for degradation.

Correct folding of relatively larger proteins in the overcrowded molecular environment in a cell is almost impossible. Therefore, Hsp70 unique structure helps the proper folding of such substrate proteins. Hsp70 can also act directly on a nascent peptide to prevent it from misfolding (17-20). Hsp70s are also involved in several other cellular processes

such as translocation across membranes, targeting proteins for degradation, and apoptosis (1, 2).

Hsp70 has several unique biochemical properties. First of all, it hydrolyses ATP at rates much lower than that of a normal ATPase. Therefore, it is not considered to be an enzyme. Hsp70-mediated protein folding rates are also much lower than those observed in cell lysate (2). In the present study, protein folding and aggregate dissolving properties of Hsp70 were investigated by employing luciferase as the substrate.

Materials and Methods

Yeast analogs of Hsp70 (Ssa1), Ydj1 (Hsp40), Hsp100 (Hsp104), luciferase assay system, and Pichia pastoris (strain GS 115) were kindly provided by Dr. Yusuf TUTAR (Cumhuriyet University). Luminometric assays were performed in a Biofix lumi-10 Macherey–Nagel luminometer as described earlier (10). Light scattering of aggregates were performed in a Shimadzu RF 5301 fluorimeter at 550 nm excitation and emission wavelength.

Results and Discussion

Luciferase is a beetle protein and produce light by the reaction below (Figure 1) (10).

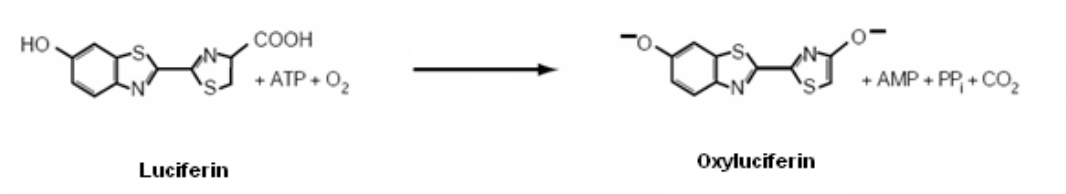

Figure 1. Luciferase enzyme forms oxyluciferin using luciferin as the substrate.

Luciferase is a good substrate to monitor the protein folding and denaturation processes. Measurements were performed using several forms of luciferase. Several forms residing between native and denatured luciferase were tested for the enzymatic activity. Light intensity correlated with degree of luciferase folding. Therefore, luciferase appeared to be a good substrate for the testing of the folder activity of heat shock proteins. Although the addition of coordinating chaperones, such as Hsp40 and Hsp100 improved the folding rate and enhanced the signal to a certain extent, addition of cell lysate caused a much more increase in the folding rate (Figure 2), probably because it included some other factors. This finding may not be surprising as several factors other than Hsp70-Hsp40 pair or Hsp100, may also affect the folding process as well. Therefore a positive control including cell lysate was also performed. Cell cytosol has unique physiological properties such as ionic concentration, macromolecular interactions and viscosity. All of these factors might affect the folding process.

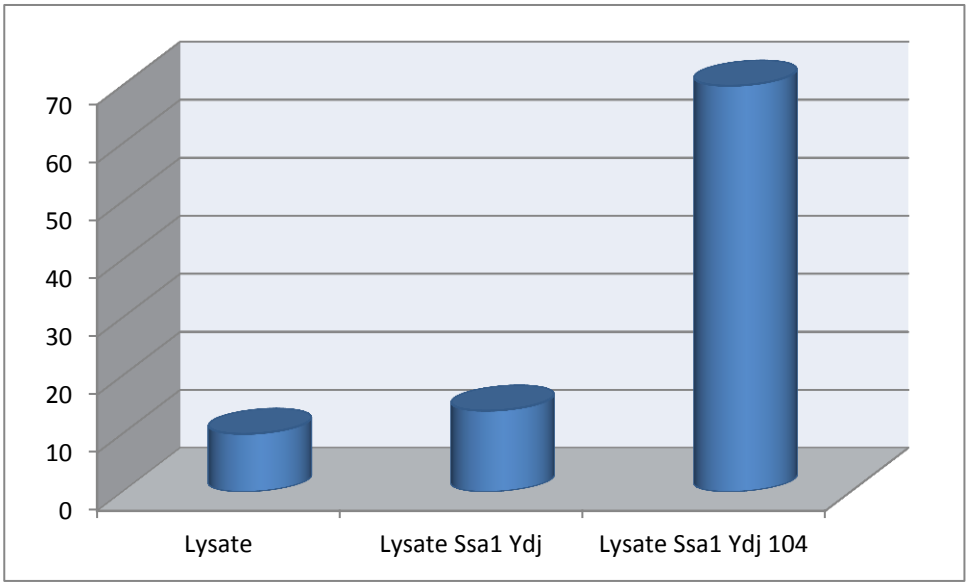

Figure 2. Luciferase folding assay in the absence and presence of Hsp70 and coordinating chaperones.

The previous experiment was designed in an aqueous environment in which heat shock proteins might easily help substrate proteins to fold. A cell cytosol has several macromolecules and at extreme conditions it can also contain protein aggregates. To mimic such an environment and to further investigate Hsp70's folder function, prevention of luciferase aggregation was measured. Luciferase was heat denatured and aliquots of a combination of Hsp70 and coordinating chaperones were added (Figure 3). Denatured luciferase formed aggregates and this helped to observe the effect of each of the added chaperone protein in the folding process of denatured luciferase. Coordinating chaperones greatly enhanced the luciferase aggregation, and thus Hsp70 had a limited effect on the dissolution of the aggregate.

Fibril formation from small aggregates appeared to make dissolving mechanism difficult. It should also be kept in mind that Hsp70-Hsp40-Hsp104 complex often has limited capacity in solubilizing substrate proteins. This complex can solve the aggregates to a certain extent. If aggregates formed fibrils, Hsp104 first breaks the fibrils and the broken fibrils are transferred to the new daughter cells (9). These data could also be supported by the weak ATPase activities of both Hsp70 and Hsp104. Therefore, it might be suggested that Hsp70 can be considered to be a "helper" rather than a "folder".

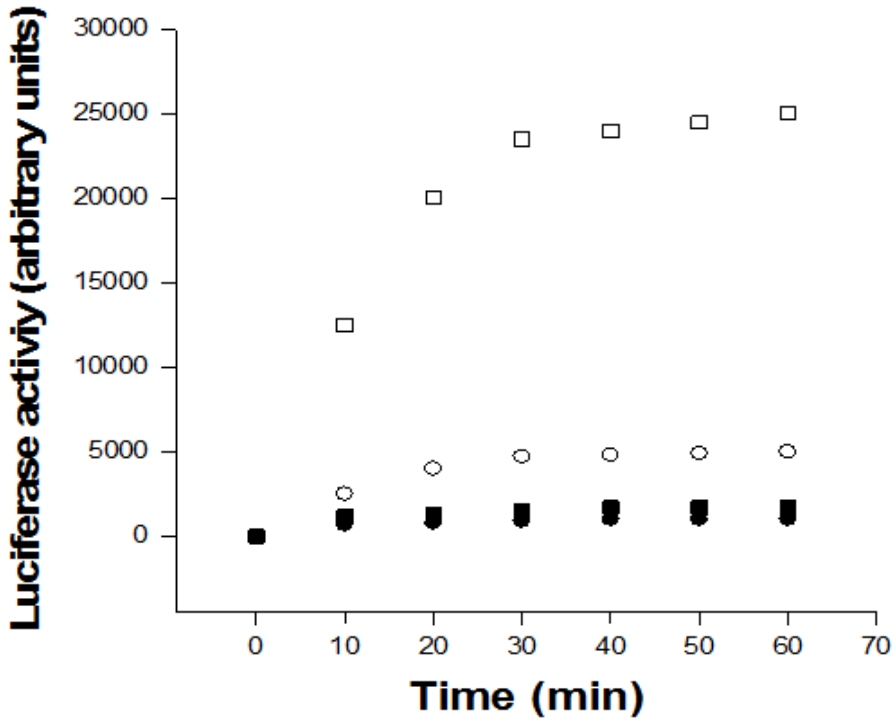

Figure 3. Luciferase aggregation prevention by Hsp70 and coordinating chaperones. Open square Ssa1, Ydj1 and Hsp104; open circle Ssa1, Ydj1; closed square Ssa1 alone, closed circle Ydj1 alone.

Conclusions

Hsp70 triggers the folding process of unfolded substrate proteins to by providing them with a closed microenvironment where hydrophobic or weak forces play a crucial role in protein-protein interaction. Coordinating and cochaperones may also contribute to the formation of such conditions. In the present work some evidence was presented evidence for the Hsp70 function both in protein folding and protein aggregation. It appeared that Hsp70 and coordinating chaperones could fold a protein substrate easily to its proper three dimensional form. However it was also evidenced that Hsp70 along with Hsp40 and Hsp100 could not solubilise aggregated substrates. Other factors must also be present because a cell lysate improved the folding of the substrate protein significantly. Thus, it can be argued that Hsp70 may not be folding the substrate but guiding it to assume its native structure.

Conflicts of interest

The authors did not declare any conflicts of interest.

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