HEAT-SHOCK RESPONSE IN THERMOPHILIC MICROORGANISMS

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Contents

Summary

In all living cells, heat shock causes the protein products of many genes to become undetectable, while greatly stimulating the production of proteins known as heat-shock proteins. The heat-shock proteins termed molecular chaperones play a major role in helping the cell to survive heating. Generally speaking, molecular chaperones are ubiquitous, constitutive proteins which couple ATP hydrolysis with the ability to assist correct protein folding and to rescue denatured proteins. A single molecular chaperone of the Hsp60 family (a chaperonin) is the protein hallmark of the heat-shock response in the extreme thermophilic microorganisms of Archaea domain. This protein has the overall structural architecture of all chaperonins (two superimposed multisubunit rings which delimitate a central chamber). However, the archaeal chaperonin shares sequence homology with the chaperonins of the eukaryotic cytosol, and constitutes together with these proteins the Group II chaperonins, whereas the chaperonins from Bacteria and eukaryotic organelles constitute the Group I chaperonins. GroEL of *Escherichia coli* (the archetype of Group I chaperonins) requires a co-chaperonin, GroES, for its function; in contrast, no GroES homologue or other partner has been identified for Group II chaperonins. *In vitro* studies clearly demonstrate that archaeal chaperonins assist protein folding at high temperature in an ATP-dependent manner, and prevent thermal denaturation of already folded proteins (the molecular mechanisms of their

activity have still to be thoroughly investigated); these experimental evidences could mirror *in vivo* involvements of the protein. Moreover, there is evidence that archaeal chaperonins form filaments at concentrations that average those in the cell; a role as elements constitutive of an archaeal "cytoskeleton" has been proposed. Whatever the physiological role(s) played by the chaperonins of thermophilic Archaea, these proteins could be employed in biotechnology as tools to rescue inactive protein material, such as the inclusion bodies.

1. Introduction

The heat-shock response was discovered as early as 1962 in the fruit fly *Drosophila melanogaster* as a dramatic change in gene activity induced by an increase of the temperature of the cultures several degrees centigrade above that optimal for growth, but below the lethal level. Later, it became clear that in different organisms heat shock causes the protein products of many genes to become undetectable, while greatly stimulating the production of other proteins, the heat-shock proteins (hsps), which are considered the hallmark of the heat-shock response.

Good evidence supports the assumption that hsps protect the cell from the effects of heat. First, the rapid and intense nature of their induction is typical for an emergency response. Second, there is a relationship between the temperature of hsps induction and the organisms environment: hsps of *Drosophila* are induced between 33° and 37 °C, the temperature of summer days; in arctic fishes growing at 0 °C, hsps are induced at 5–10 °C; in mammals, hsps are induced by fever temperatures. Third, the deletion of the genes that encode some hsps renders the organism unable to survive a heat-shock challenge.

Thus, heat shock inactivates or down-regulates many genes but activates others, whose function is to help the cell to survive.

1.1. The Concept of Molecular Chaperone

Some hsps are those known as molecular chaperones. The exact cellular role of these hsps has remained obscure for a long time; it is now well established that molecular chaperones play a role in maintaining the cellular proteins in active form. Even if not all hsps are chaperones (and, *vice versa*, not every molecular chaperone is a hsp), this group of hsps received much attention due to the relevant roles they exert in the cell. Molecular chaperones are ubiquitous, multifunctional, and essential proteins, since they play several vital roles in all cell types, cell compartments, and organelles.

The temperature causes various forms of damage on proteins, in the test tube (*in vitro*) as well as in the cell (*in vivo*); some damages are as severe to cause the inactivation (loss of biological activity) of the proteins. *In vitro*, many proteins lose their native, functional conformation upon heating; some of them tend to aggregate; the process may become irreversible and generalized within the cell, which ultimately dies. Thus, the survival of an organism to a heat shock depends on its capability to preserve the functionality of its proteins.

Many molecular chaperones also function in the absence of heat shock, namely, under normal physiological conditions. Molecular chaperones, in fact, play critical roles in physiological growth conditions: they help cellular proteins to fold correctly during synthesis on the ribosome, after translation and denaturation.
A polypeptide that emerges from the ribosome or crosses a membrane is subject to contact with other protein domains, either intra- or interspecific, because of the high cytosolic protein concentration. These contacts are mainly due to the interactions among hydrophobic surfaces or groups that are still solvent-exposed in a protein with a nonnative conformation; heating causes the exposure of the internal hydrophobic portions of proteins, and strengthens the hydrophobic interactions.

In summary, the folding of a protein is an event with a major pitfall along the way, even during normal growth: protein aggregation. There is clearly a need for mechanisms avoiding aggregation and subsequent misfolding.

In vitro experiments with model proteins and peptides demonstrated that molecular chaperones do not bind to native proteins, rather they interact with unfolded or partially folded protein molecules without sequence or structure specificity. Thus, the formation of a complex between the chaperone and a non-native protein prevents aggregative events. The molecular chaperones are proteins constituted by domains with different functions; most of them are ATPases that couple the hydrolysis of the nucleotide with the capability to renature (reactivate) the nonnative protein bound.

The discovery of molecular chaperones does not contradict the successful *in vitro* renaturation of denatured and reduced ribonuclease obtained by Anfinsen in the absence of any other component (this result led to the conclusion that the primary sequence of a protein dictates its three-dimensional structure). In fact, it is known that many small one-domain proteins do not need assistance from chaperones in the cell to correctly fold, whereas a long polypeptide with more than one domain undergoes chaperone-assisted folding.

2. Even Extreme Thermophiles Display Heat-Shock Response

The cell response to an upshift in temperature consists in increased synthesis of a defined set of cellular proteins. An interesting question is whether or not organisms that normally grow at very high temperatures exhibit such a response.

With some exceptions, the microorganisms that live at and above 70 °C (the extreme thermophiles) belong to the kingdom Crenarchaeota in the domain Archaea. The Crenarchaeota are considered the most ancient living cells; a number of vital mechanisms are still not understood in such microorganisms.

Sulfolobus sp. are extreme thermophilic crenarchaea which live in the acidic hot springs of volcanic areas. The study of heat-shock response in these microorganisms produced important results. In one typical experiment, *Sulfolobus shibatae* cells were grown up to the late exponential phase at the optimal temperature of 70 °C; then, while a sample was maintained at the same temperature as a control, others were heated to 88 °C for various periods of time before being challenged at 92 °C, the lethal temperature for the

archaeon. No survival at 92 °C was observed when cultures were preheated to 88 °C for 15 or 30 min; enhanced survival (up to 2 hours at 92 °C) was detectable in those cultures that were preincubated at 88 °C for 60 min or longer. The experiments show that the thermal resistance of thermophiles that normally thrive at high temperatures can be increased by a heat shock. This phenomenon could be observed in all living cells, and is termed acquired thermotolerance.

Changes in protein synthesis can be easily monitored by *in vivo* labeling. Cells are grown in the presence of a radiolabeled amino acid (usually L-^{35}S-methionine or L-^{14}C-labeled amino acids) which will be incorporated in newly synthesized proteins; the extracts prepared from the cultures are subjected to polyacrylamide gel electrophoresis in sodium dodecylsulphate (SDS-PAGE), a technique which separate the subunits constituting the proteins on the basis of their size; the exposure of the gel to an X-ray film (autoradiography) reveals the proteins which incorporated the labeled amino acid; a densitometer scan allows quantitative calculations. This technique made it possible to conclude that heat shock in *S. shibatae* stops detectable synthesis of most cellular proteins and causes preferential synthesis of primarily one protein whose subunit mass averages 60 kDa and which possesses ATPase activity. This single protein is induced by heat shock in different extreme thermophilic crenarchaea. This protein represents one of the most abundant proteins present in the cytosol of not-shocked cells, which argues in favor of its important physiological role. The protein hallmark of the heat-shock response in extreme thermophilic archaea belongs to the Hsp60 family of molecular chaperones whose members are called chaperonins. The chaperonin of *Pyrodictium* represents 80% of total protein amount in cells which were grown at 108 °C (2 °C above the maximal temperature); this allows the microorganism to survive one hour in the autoclave.

3. Archaeal Chaperonins

Chaperonins of all living cells have a complex quaternary structure: two rings of about 60 kDa-subunits each are stacked face to face with a central cavity which accommodates the polypeptide that needs help to fold. The overall structure resembles a barrel. Chaperonins isolated from *Escherichia coli* (termed GroEL), mitochondria, and chloroplasts have seven identical subunits per ring, and share high sequence similarity. Sequence-related proteins consisting of a single homo-oligomeric ring of seven subunits of ~10 kDa each have been isolated together with these chaperonins; these molecules (termed co-chaperonins) are regulators of the chaperonins' action. The chaperonin complex of *E. coli* (the chaperonin GroEL and the co-chaperonin GroES) is very well known. GroES completes the functional chaperonin by serving as a lid to occlude one of the two ends of the barrel formed by the GroEL rings.

The crystal structure determination of GroEL revealed three domains in each subunit: the apical domain faces the central cavity, and its flexible regions are involved in the binding of the polypeptide substrate and the co-chaperonin GroES; the equatorial domain provides most of the intratoroidal side-to-side contacts and all the ring-to-ring contacts, and contains the ATP-binding site; the domain intermediate between the other two probably allows allosteric domain movements. Electron microscopy and crystallography studies showed that in the *E. coli* system the barrel of GroEL would be

alternatively open (when the polypeptide enters the barrel) or closed (folding stage) when the GroES ring attaches to one of the ends of the barrel and covers it as a lid. While the GroEL barrel is closed, the inside of its wall changes from hydrophobic to hydrophilic, owing to conformational changes induced by the binding of ATP and the GroES ring to the GroEL barrel. In this stage, the polypeptide finds conditions that prompt it to bury hydrophobic residues to stay in solution, hence, to fold. The next step is the opening of the barrel by ATP-driven release of the GroES ring and the exit of the folded polypeptide. The affinity of GroEL for the substrate protein is allosterically regulated by the nucleotide: in the absence of nucleotide or in the presence of ATP binding, the chaperonin exists in the "high affinity" conformation for the protein; upon hydrolysis of ATP, the chaperonin adopts the "low affinity" conformation for the protein which is released; the binding of the nucleotide drives the chaperone to the conformation able to enter another folding cycle; several rounds of binding and release of the protein upon expenditure of energy could be required to reach the folded state. Cryoelectron microscopy and computer-assisted image reconstruction permitted to visualize the morphologic changes that the complex undergoes when it passes from the ADP- to the ATP-bound stage. A gallery of images of *E. coli* GroEL alone and in complex with GroES, and movies of the allosteric changes of the complex upon nucleotide binding/hydrolysis can be found in the site <http://bioc09.uthscsa.edu/~seale/Chap/struc.html>.

Chaperonins isolated from Archaea display the typical double-ring structure of all chaperonins. However, more than seven subunits occur in each ring: eight subunits are present in the chaperonins from *Pyrodictium occultism* and *Thermoplasma acidophilum*, and nine in the chaperonins from *Sulfolobus shibatae* and *Sulfolobus solfataricus*. Moreover, the occurrence of two or three nonidentical subunits was described. Most remarkably, the primary structure of chaperonins from archaea shows no significant relationship to GroEL-like chaperonins, but nearly 40% identity with the chaperonin present in the eukaryotic cytosol (termed TCP-1, TriC, or CCT) which is involved in the biogenesis of two proteins found in the cytoskeleton, tubulin and actin. Therefore, based on sequence data, the chaperonins from archaea together with the chaperonins of eukaryotic cytosol constitute the Group II chaperonins, distinct from the Group I present in Bacteria, mitochondria, and chloroplasts. It should be noted that the organelles of the eukaryotic cell are the descendants of endosymbiotic bacteria. An important difference between Group I and Group II chaperonins is that co-chaperonins have not been identified for Group II chaperonins.

Crystallographic analyses showed that in *T. acidophilum* chaperonin the central cavity seems to be closed or contracted upon ATP hydrolysis. Functional studies showed that the *Sulfolobus solfataricus* chaperonin cycles between two ATP-dependent conformational states: in the absence of the nucleotide, the chaperonin exists in a conformation that is able to bind an unfolded protein; ATP hydrolysis causes protein release in folded form and the rearrangement of the chaperonin in a conformation which is unable to bind the protein molecule; ATP binding triggers the chaperonin again in the conformation with high affinity for the protein. Thus, the knowledge that the affinity for the protein is regulated via ATP-induced conformational changes applies also to archaeal chaperonins.

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Biographical Sketches

Mosè Rossi received the "Laurea" in Chemistry in 1961 from University of Naples in Italy. In the years from 1962 to 1964, he was a research fellow first of the U.S. National Institute of Health and then of the Italian National Research Council (CNR). From 1964 to 1969, was a staff scientist at the International Institute of Genetic and Biophysics in Naples. In this period, he worked on DNA synthesis in sea urchin embryo development and on the regulation of the activity of the allosteric enzyme dCMP aminohydrolase enzyme discovered in Naples. From 1970 to the end of 1972, he was P.H.S. visiting scientist in Prof. D.O. Woodword's laboratory at the Department of Biological Sciences, Stanford University, California (USA) and spent three months at McGill University, Montreal, Canada, in the Cancer Research Unit. From 1972 to 1980, he was Associate Professor of Enzymology at Faculty of Science, University Federico II of Naples and since 1981 Full Professor of Enzymology in the same Faculty. In 1985, he was nominated Director of the Institute of Proteins Biochemistry and Enzymology of CNR, position held also actually. Since 1982 his research work has focused on molecular adaptation of different microorganisms at high temperature and, in particular, on the molecular basis of thermostability and thermoactivity of several enzymes isolated from such microorganisms. He is author of 230 original publications.

Annamaria Guagliardi graduated in Biology at the University of Naples in 1984. In 1991, she became a researcher of the University of Naples in the Department of Biological Chemistry. She was responsible for the Project Biotechnology "Employ of chaperones from thermophilic archaea in the regain of biological activity of denatured proteins" of CNR. She studied different enzymes form thermophilic sources, including dehydrogenases, redox-proteins and proteins involved in the control of protein homeostasis (molecular chaperones and proteases).