Hsp70 chaperone homolog in *Synechocystis* PCC6803: the role of DnaK2 in defence against different stresses and in modulation of the physical state of tilakoid membranes

Ph.D. thesis

Viktória Varvasovszki

Supervisor: László Vígh, Ph.D., D. Sc.

Hungarian Academy of Sciences
Biological Research Center,
Institute of Biochemistry,
Laboratory of Molecular Stress Biology

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Introduction

Living organisms have the capability to adapt to sudden changes in their environment. Since most of these stimuli (temperature up- and downshift, UV-B radiation, etc.) have detrimental effects on cells, they had to develop adequate protective systems. One of these general systems is the network of molecular chaperones, the so-called stress proteins. **Molecular chaperones are specialized and universally conserved proteins that bind nonnative states of other proteins and assist them to reach a functional conformation, in most cases through the expenditure of ATP** (Ellis, 1997). Chaperones function not only during the normal operation of cellular processes but in addition act to limit damage caused by stresses such as heat shock. DnaK proteins of prokaryotic cells belong to the Hsp70 chaperone family which is one of the highly conserved ubiquitous groups of heat shock proteins. The members of the Hsp70 chaperone machine are DnaK (70 kDa), DnaJ (40 kDa) and GrpE (20 kDa). These chaperones act together co-operatively with the Hsp60 chaperonin family during the process of assisted protein folding. The Hsp70 proteins play important role in both stress or non-stress conditions. Generally, they are involved in protein translocation across cytoplasmic membranes, protein degradation and induced by several different stress conditions, such as temperature and osmotic damages (Georgopoulos, 1993).

Our model system is the unicellular cianobacterium, ***Synechocystis* PCC6803** has been used extensively as a powerful model for studying the molecular mechanisms of stress response in photosynthetic organisms. Recently, the complete nucleotid sequence its genome and the potential protein coding regions have been determined (Kaneko, 1996). On the basis of this genomic map and in contrast to many other species, there are three *dnaK*s (as we designated them *dnaK1*, *2* and *3*), four *dnaJ* genes and only a single copy of *grpE* in this organism.

It is noted, that three *dnaK* homologs were observed in the genome of another cyanobacterium, *Synechococcus* PCC7942, all exhibiting high similarity to each of the three *Synechocystis dnaK*s. Of these three homologues *dnaK2* and *dnaK3* were essential in *Synechococcus*, but only *dnaK2* displayed a typical heat shock response (Nimura, 2001). DnaK1 and DnaK2 proteins were detectable mainly in the cytosolic fraction, and a significant amount of *Synechococcus* DnaK3 was localized to the thylakoid membrane (Nimura, 1996). A *dnaJ* homolog of *Synechococcus* is located immediately downstream of *dnaK3* (as in *Synechocystis*) and the two genes seemed to be co-transcribed. Moreover, since the DnaJ protein, which is essential for growth even under normal conditions, is also located mainly in the thylakoid of this cyanobacterium, a specific, photosynthesis-related function has been assigned to the membrane-colocalized DnaK3 and DnaJ, in which they presumably cooperate (Ouguchi, 1997). Pairing of the membrane-associated DnaJ-DnaK tandem with physiological relevance is further supported by the finding that the DnaJ-like DjlA, associated with the inner membrane, has been identified as a DnaK co-chaperone of *E. coli* (Genevaux, 2001).

The physical state of cell membranes is known to be a very sensitive monitor of the most diverse environmental changes. This feature was suggested to render cell membranes an ideal location for the primary temperature stress sensor (Vigh, 1993; Carratù, 1996). Strongly supporting this hypothesis, catalytic hydrogenation of unsaturated lipids in the surface membrane of cyanobacterial cells at constant temperature

resulted in the activation of transcription of a desaturase gene (Vigh, 1993), which normally is induced by cold stress (Los, 1993). The rapid increase of the membrane fluidity induced by the direct physical effect of the temperature upshift during heat shock is well documented (Mejia, 1995; Dynlacht, 1992). On the other hand, all organisms examined to date produce the evolutionary conserved HSPs when they are exposed to a sudden, sublethal increase in the ambient temperature. **If the primary heat shock sensor is membrane associated, one can suppose that any modification of membrane physical properties analogous to heat-induced perturbations also could lead to changes in the level of expression of heat shock genes.** Further supporting the view that thermal stress is transduced into a cellular signal at the level of membrane, in parallel with the adaptive readjustment of membrane fluidity, the treshold induction temperature of HSPs was shown to be subject of temperature acclimation in various organisms (Piper, 1995; Dietz, 1992; Lehel, 1993).

It was un intriguing finding of our workgroup that the heat shock proteins GroESL of *Escherichia coli* and Hsp17 of *Synechocystis* proved to be thylakois-associated during heat shock (Horváth, 1998; Török, 2001). We also have shown in a model using lipid membranes and active GroESL oligimers that the chaperone binding to lipids is governed by the composition and physical state of the host membranes. Chaperones, wich associated with unilamellar vesicules and stabilized the membranes at high temperature by increasing its microviscosity, retained their capability to assist protein folding (Török, 1997). The small heat shock protein Hsp17 of *Synechocystis*, inducible by heat and ethanol, also was found to be pherifericallyassociated with the membrane in *Leuconostoc oenos* (Jobin, 1997).

We assume that the ability of HSPs to alter membrane organization and physical parameters is a rapid, reversible and powerful tool of cellular adaptation. It may antagonize the heat-induced lipid disorganization of the membrane and thus might serve to preserve membrane structure and function during heat stress (Vigh, 1998). Furthermore, the association of HSPs with membranes likely causes inactivation of the membrane perturbation signal induced by heat, thereby turning off the heat shock genes in a feedback loop. The modulation of membrane physical order may repress transcription of heat shock genes in the heat-modified-state, explaining the known temporality of induction of the stress response. Therefore, such proposed “cross-talk” between the membrane located sensor and the heat shock response suggests the existence of a feedback mechanism of heat shock gene regulation (Vigh and Maresca, 2002).

Specific aims

Previously, our laboratory has been studying the Hsp60 (chaperonin) family with regard to the function of these proteins in the defence of biological membranes during heat stress. Setting out from that generally accepted view that in the diversified world of stress proteins the role of the chaperone families are closely related to each other, the subject of this thesis was **the investigation of the members of another universal group of chaperones, the Hsp70-family in *Synechocystis* PCC6803.**

In this direction our specific aim was to

1. Test whether the *hsp70*-, *hsp40*- and *hsp20* homolog ORFs found on the genetic map of *Synechocystis* PCC6803 have transcriptional activity on basal level and during heat stress.

2. Obseve that the protein products of the heat-inducible chaperone genes localize wich parts of the cell and whether they are bound to the membranes.

3. Clone viable *hsp70*-deficient mutant *Synechocystis* strains.

4. Show if the inactivation (even partial) of a *hsp70* homolog gene in *Synechocystis* has influence on the transcriptional or translational expression of other chaperone family members.

5. Clarify the nature of physiological changes caused by different stresses (as heat and cold stress, photoinhibition or UV-B radiation) and their combination in the membrane-related photosynthetic processes.

6. Reveal whether the amount of the Hsp70 homolog proteins in *Synechocystis* cells has any influence on the physical composition or state of the thylakoid membranes.

Summary of the results

We examined the expression and the function of the Hsp70 homolog DnaK chaperone family in the photoautotropic cyanobacterium, *Synechocystis PCC6803.*

1. We have established that, unlike in *Synechococcus*, **only one of the three *dnaK* genes, *dnaK*2, is transcribed and it exhibits a typical heat stress response in *Synechocystis* PCC6803**. Northern experiments, primer extension reactions and RT-PCR analysis were carried out with specific probes for ORFs, but we failed to detect any transcripts corresponding to either *dnaK1* or *dnaK3,* under either normal or heat-shock conditions. To our surprise, **together with *grpE*, all *dnaJ* homolog genes are expressed constitutively** and appear to be uninducible by high-temperature stress.
2. According to the Western analysis, we have shown that at normal temperature, **the DnaK2 protein is located both in the cytoplasm and the thylakoid membrane fraction.** Under heat shock conditions, the expression of this chaperone is significant and a well-defined amount of DnaK2 becomes membrane-bound.
3. We have prepared **a partially *dnaK2*-deficient merodiploid *Synechocystis* strain** using the method of site-directed insertional mutagenesis, since we could not disrupt all copies of *dnaK2* in *Synechocystis*, which strongly suggests that **this gene is essential for growth under normal conditions.**
4. Following the photosynthetic oxigene evolution of the different strains, we have proved that the **partially DnaK2-deficient cells displayed temperature sensitive phenotype, but were able to acquire thermotolerance.**
5. The **study of the photoinhibition of the mutant and wild type *Synechocystis* cultures** adapted different growth temperatures has given the following results:
   - **In case of the cells grown at 30°C**, the high light treatment alone did not cause considerable changes in the photosynthetic activity of the different cultures. There was a significant decrease in this parameter in case of both strains when they were subjected to 22°C.
   - **Both cultures adapted to 36°C** have shown the photoinhibition effect even at their growth temperature.
   - **Strains cultured at 22°C** tolerated the high light treatment less at their growth temperature than at 30°C. They both showed decreased oxigene-evolution when they were returned to normal light conditions.
6. According to **the analysis of UV-B tolerance**, we have established that the photosynthetic oxigen-evolution of the *dnaK2* mutant cells dropped to the 40% of the normal level and it was decreasing also during the recovery process and the mutant cells are finally died. In contrast, the wild type strain have been almost completely recovered.
7. The result of the experiments concerning the study of the changes in the membrane physical state using the method of fluorescence-anisotropy showed that **thylakoid membranes derived from the *dnaK2* mutant cells are always more rigids, than these of the wild type strain,** regardless the temperature preadaptation.
8. The analysis of the fatty acid composition of the thylakoid membranes has revealed **a significant increase in the level of oleic acid (18:1) in the lipids of the mutant cells** in comparison with the wild type strain, while the amount of the linolic acid and the γ-linoleic acid is diminished.

In conclusion, as the photosynthesis of the *dnaK2* mutant is more sensitive to high temperature, but is able to acquire thermotolerance, this might suggest that a specific composition and specific levels of stress proteins are a prerequisite of effective stress defense, but the heat-adaptation process is affected less strongly. Additional factors, such as remodeling the composition and physical state of the membranes are likely to act to explain the remaining capacity of partial *dnaK* mutants to develop thermotolerance by preadaptation.
According to the results of this thesis, we could prove at the first time that **an organism deficient in a basic heat shock protein, the DnaK, is a membrane-mutant** at the same time.