Heat shock response in hyperthermophilic microorganisms

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ABSTRACT

Hydrothermal environments contain steep thermal gradients and variable temperature conditions. Hyperthermophilic microorganisms, those which grow at temperatures exceeding 90°C, are common in these environments and have numerous means for tolerating hyperthermal stress. All hyperthermophiles examined produce a heteromeric chaperonin complex which is the primary protein produced during heat shock, though other proteins of unknown function are also produced. Furthermore, many hyperthermophilic proteins demonstrate sufficient intrinsic thermostability to withstand brief periods of hyperthermal stress. These organisms also produce putative thermoprotectants, such as di-*myo*-inositol phosphate (DIP) and cyclic diphosphoglycerate (cDPG), which may stabilize many hyperthermophilic proteins at super-optimal temperatures. Hydrostatic pressure also enhances the thermotolerance of hyperthermophiles and their proteins *in vitro* during exposure to heat-shock temperatures. The specific activity of six metabolic proteins remains constant during heat shock in *Pyrococcus* sp. strain ES4 indicating *in vivo* protein stabilization during periods of hyperthermal stress.

Introduction

In general, exposure to super-optimal temperatures induces what is known as the 'heat-shock response', which is the synthesis of enzymes that function primarily to prevent protein aggregation, to reassemble damaged proteins, and to degrade those proteins which are beyond repair [33]. While the importance of intrinsic factors in cellular stability has been recognized for almost a decade, it is only recently that extrinsic factors such as hydrostatic pressure and biofilm formation have also been found to enhance tolerance to hyperthermal stress.

Hyperthermophilic microorganisms are defined as those organisms which grow at 90°C or higher and have the highest growth temperatures known for life [3]. Thus the following questions arise with regard to hyperthermophiles and heat shock. How do hyperthermophiles respond to super-optimal temperatures and hyperthermal stress? Do hyperthermophiles constitutively express heat-shock proteins, or are their heat-shock proteins regulated? Do hyperthermophiles contain any novel heat-shock response mechanisms? This article will review the current knowledge of heat shock in hyperthermophilic microorganisms. Other reviews of heat shock in hyperthermophiles are also available [see 3,44]. Within hydrothermal environments, a native habitat of

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hyperthermophiles, temperatures are prone to spatial and temporal variations due to tidal flexing of the earth's crust which causes diurnal temperature fluctuations [42], dynamic fluid flow patterns, and steep temperature gradients. Therefore, hyperthermophiles must employ thermal stress mechanisms to withstand the super-optimal temperatures encountered from these variations.

In vivo evidence for heat shock in hyperthermophiles

When a culture of microorganisms is exposed to super-optimal growth temperatures, the number of viable cells decreases exponentially with time, and this rate of decline increases exponentially with increasing temperature [32]. However, if a culture is exposed to a mild hyperthermal stress temperature prior to exposure to a more lethal temperature, then the number of viable cells in the culture will remain significantly higher for a period of time before they begin to die. This kinetic display of enhanced tolerance to super-optimal temperatures is known as 'acquired thermotolerance' and is attributed to the expression of the heat-shock response [33]. Acquired thermotolerance kinetics have been observed in the sulfur-oxidizing hyperthermophile *Sulfolobus shibatae* and in the anaerobic sulfur reducer *Pyrococcus* sp. strain ES4 [13,47]. ES4 grown at 95°C (optimum growth at 99°C) demonstrated acquired thermotolerance kinetics at 105°C when cultures were exposed to 102°C for 90 min prior to the shift to 105°C [13].

In both *S. shibatae* and ES4, proteins were produced during exposure to a mild super-optimal temperature, though the overall rate of protein synthesis decreased [47, J Holden and J Baross, unpublished results]. The densest protein band produced in both organisms during heat-shock, as seen by pulse labeling with ^{35}S-labeled amino acids, had a mass of approximately 60 kDa and was shown to be a chaperonin [see below]. In ES4, other proteins of various masses were also produced whose function remains unknown. Furthermore, during *in vivo* exposure to 102°C for up to 90 min, six metabolic proteins maintained constant specific activity [J Holden and MWW Adams, unpublished results] demonstrating that some factor(s), extrinsic or intrinsic, was protecting these proteins at the super-optimal temperature for this organism.

Hyperthermophilic chaperones

The most abundant protein produced during the heat shock response of hyperthermophiles is the TF55 chaperone. This well-studied enzyme is composed of two stacked rings made of either eight or nine protein subunits per ring. The enzyme is a hexadecamer in *Pyrodictium occultum* [34,35] and an octadecamer in *S. shibatae* [25,46], these are heteromeric proteins, and consist of one of two closely related proteins, each with a mass of approximately 60 kDa, which are present in a 1:1 ratio [16,35]. This chaperone is expressed constitutively and is abundant in hyperthermophiles under normal growth conditions, suggesting that it is used to protect proteins (perhaps intermediates formed during translation) from high temperatures when the cell is not under duress. The TF55 chaperone, a homolog of the enzyme (as detected by antibodies), or a homolog of the TF55 gene, has been found in all of the major genera of hyperthermophilic archaea [2,16,17,34,48]. The chaperone from *Sulfolobus* spp. reduced the denaturation rate of a target protein at high temperatures [46] and hydrolyzed ATP in the presence of K^+ [10,20]. The chaperone from *S. solfataricus* underwent a major conformational change in the presence of ATP and Mg^{2+}, bound to denatured target protein with refolding occurring

upon addition of K^+, and released the substrate following hydrolysis of the ATP [10]. Upon ATP hydrolysis, the chaperone from *S. shibatae* dissociated into free subunits and the equilibrium between complex and subunits was affected by temperature and ATP levels [37]. There was an 80% recovery of the activity of heat-denatured lysozyme when incubated with *S. solfataricus* chaperone, Mg^{2+}, K^+, and ATP [10]. Recently, it has been suggested that TF55 is also a component of archaeal cytoskeleton [45] and is most closely related phylogenetically to TCP-1 cytoskeleton protein from mice [16,46]. The protein shows very little phylogenetic homology with other known mesophilic chaperones, but is considered part of the GroEL/HSP60 chaperonin family based on functional and structural similarities [16,44].

Conspicuously absent from hyperthermophilic archaea is a homolog to the other major heat-shock chaperone typically found in mesophiles, the HSP70/DnaK protein. No *dna*K homolog has been found in the genome sequences of *Methanococcus jannaschii, Archaeoglobus fulgidus, Pyrobaculum aerophilum*, *Pyrococcus horikoshii*, or *Pyrococcus furiosus* [4,9,18,19, R Weiss, personal communication], and Southern blotting using a *dna*K probe from *Methanosarcina mazei* S-6, a mesophilic archaeon, against genomic DNA from *Methanothermus fervidus, M. jannaschii,* and a *Sulfolobus* sp. failed to detect a homolog [23]. Little is known about the heat-shock response of hyperthermophilic eubacteria; however, homologs of *dna*K and *gro*EL are present in both the *Thermotoga maritima* and *Aquifex aeolicus* genomes [7, J Holden, www.ncbi.nlm.nih.gov] suggesting that the heat-shock responses in these organisms are a high-temperature analog of the mesophilic response.

Other extrinsic factors which lead to thermotolerance

In addition to chaperones, extrinsic non-enzymatic factors, such as stabilizing solutes (or thermoprotectants), biofilm formation, and hydrostatic pressure might enhance the stability of hyperthermophiles at super-optimal temperatures. High concentrations of cyclic 2,3-diphosphoglycerate (cDPG) were found in *Methanothermus fervidus* and *Methanopyrus kandleri* [12,22] and high concentrations of di-*myo*-inositol phosphate (DIP) were found in *Methanococcus igneus* (but not in other *Methanococcus* spp.), *Pyrococcus woesei*, *P. furiosus*, *Pyrodictium occultum*, *A. fulgidus*, and *T. maritima* [6,27,38]. *In vitro* experiments showed that cDPG acted as a stabilizer of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and malate dehydrogenase at denaturing temperatures, but not of DNA [12], and that DIP also stabilized G3PDH *in vitro* [41]. However, DIP from *T. maritima* did not stabilize hydrogenase or pyruvate ferredoxin oxidoreductase suggesting that it is not a general thermoprotectant [38]. The concentration of cDPG increased in *M. fervidus* with increasing growth temperature [12], and the concentration of DIP increased up to 20 fold after exposure to super-optimal temperatures in *M. igneus*, *P. furiosus*, and *A. fulgidus* [6,27,28]. Therefore, these data suggest that: a) cDPG and DIP are part of the heat-shock response in hyperthermophiles; b) they act specifically to stabilize certain proteins rather than other macromolecules; and c) the enzymes necessary for their synthesis may be produced during heat shock.

Biofilms composed of acidic exocellular polysaccharides were formed by the hyperthermophilic archaea *Thermococcus litoralis*, *A. fulgidus*, and *M. jannaschii* [24,39]. *A. fulgidus* cultured under optimal growth conditions do not form a significant biofilm;

however, after exposure to super-optimal temperatures, cultures produced a significant biofilm [24]. The biofilm was enriched in proteins which were present in low levels within free-living cells, and the genes for these proteins and for biofilm production may also be induced during heat shock in hyperthermophiles. The function of biofilm formation by hyperthermophiles during heat stress is not known.

Hydrostatic pressure appears to be a non-inductive form of protein stabilization in hyperthermophiles. Pressure increased by 2-3 °C the maximum growth temperatures of *Desulfurococcus* sp. strain YM, *Thermococcus* sp. strain ES1, *T. peptonophilus*, *Pyrococcus* spp. strains ES4 and GDB, *P. abyssi*, and *M. jannaschii* [5,15,26,31,36]. ES4 demonstrated enhanced thermotolerance to super-optimal temperatures at high pressure (22 Mpa) even when the cultures had been grown at low pressure (3 Mpa), and decompression of cultures during heat shock did not result in the loss of this enhanced thermotolerance, suggesting that pressure left a lasting effect on the conformation of the pressure-affected compounds [14]. Furthermore, the heat-shock protein patterns in ES4 were formed at a higher temperature in cultures that had been subjected to heat shock under pressure. *In vitro* experimentation has shown that pressure enhanced the thermostability of DNA polymerase, hydrogenase, α-glucosidase, and a protease [11,29,30,43]; however, this effect is not general for hyperthermophilic proteins, as adenylate kinase from *M. jannaschii* was not stabilized by pressure [21].

Intrinsic factors

In addition to extrinsic factors which yield stability to proteins at super-optimal temperatures, many hyperthermophilic proteins are capable of withstanding these temperatures due to the inherent thermostability of the protein [1]. *In vitro* thermostability experiments with glutamate dehydrogenase from ES4 showed the enzyme had a half-life of 10.5 and 3.5 h at 100 and 105°C, respectively [8], and the half-life at 100°C of GDH from *P. furiosus* decreased from 10 to 2.3 h when the protein concentration was diluted 20 fold suggesting that even neighboring proteins may act as an extrinsic thermostabilizing factor in the cytosol [40]. Therefore, though the organism may experience thermal stress at a given temperature, it appears that many enzymes and structural macromolecules are capable of withstanding these temperatures and the heat-shock response is set to respond to a certain subset of temperature-sensitive proteins.

Conclusions

The primary, and possibly only chaperone produced in hyperthermophiles is expressed constitutively and is the major protein produced during heat shock. Its constitutive expression and high abundance during normal growth suggests that the chaperone is important for stabilizing protein intermediates at all growth temperatures. Hyperthermophilic archaea are unique in that they do not appear to produce any chaperones which are homologous to the highly conserved GroEL/HSP60 and DnaK/HSP70 proteins found in bacteria and eukaryotes. However, heat shock in hyperthermophiles is not limited to the production of the TF55 chaperone as other proteins are produced during heat shock. These other proteins may be involved in the synthesis of thermoprotectants (i.e., cDPG and DIP) and protective biofilms or may have other functions (i.e., proteolytic activity). The production of high levels of cDPG and DIP during hyperthermal stress appears to be unique

to hyperthermophiles. Furthermore, other factors such as hydrostatic pressure and intrinsic protein stability are important in assessing the overall response that hyperthermophilic proteins have to super-optimal temperatures. These mechanisms may be employed regularly by hyperthermophiles *in situ*, given the thermally dynamic character of their native hydrothermal habitats.

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