

Thermodynamics of Heat Shock Response

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July 26, 1999

Abstract: Production of heat shock proteins are induced when a living cell is exposed to a rise in temperature. The heat shock response of protein DnaK synthesis in *E.coli* for temperature shifts $T \rightarrow T + \Delta T$ and $T \rightarrow T - \Delta T$ is measured as function of the initial temperature T . We observe a reversed heat shock at low T . The magnitude of the shock increases when one increase the distance to the temperature $T_0 \approx 23^\circ$, thereby mimicking the non monotonous stability of proteins at low temperature. This suggest that stability related to hot as well as cold unfolding of proteins is directly implemented in the biological control of protein folding.

PACS numbers: 5.65.+b, 82.60-s, 87., 87.10.+e, 87.14.Ee, 87.15.-v, 87.17.-d

Chaperones direct protein folding in the living cell by binding to unfolded or misfolded proteins. The expression level of many of these catalysts of protein folding change in response to environmental changes. In particular, when a living cell is exposed to a temperature shock the production of these proteins are transiently increased. The response is seen for all organisms [1], and in fact involves related proteins across all biological kingdoms. The heat shock response (HS) in *E.coli* involves about 40 genes that are widely dispersed over its genome [2, 3]. For *E.coli* the response is activated through the σ^{32} protein. The σ^{32} binds to RNA polymerase (RNAP) where it displaces the σ^{70} subunit and thereby changes RNAP's affinity to a number of promoters in the *E.coli* genome. This induce production of the heat shock

proteins. Thus if the gene for σ^{32} is removed from the *E.coli* genome the HS is suppressed [2, 4] and also the cell cannot grow above 20°C .

The HS is fast. In some cases it can be detected by a changed synthesis rate of *e.g.* the chaperone protein DnaK already about a minute after the temperature shift. Given that the DnaK protein in itself takes about 45 seconds to synthesize the observed fast change in DnaK production must be very close to the physical mechanism that trigger the response. In fact we will argue for a mechanism which does not demand additional synthesis of σ^{32} in spite of the fact that DnaK is only expressed from a σ^{32} promoter, and thus postulate that changed synthesis of σ^{32} only plays a role in the later stages of the HS. To quantify the physical mechanism we measure the dependence of HS with initial temperature and find that the magnitude of the shock is inversely proportional to the folding stability of a typical globular protein.

The present work measure the expression of protein DnaK. Steady state levels at various temperature and growth conditions can be found in [5, 6]. The steady state number of DnaK in an *E.coli* cell varies from approx. 4000 at $T = 13.5$ to approx. 6000 at 37°C , thereby remaining roughly proportional to number of ribosomes. DnaK is a chaperone, and have a high affinity for hydrophobic residues [7], as these signal a possible misfold (for folded proteins the hydrophobic residues are typically in the interior). σ^{32} control the expression of DnaK, the σ^{32} must bind to the RNAP before this bind to the promoter for DnaK. One expect at most a few hundred σ^{32} in the cell, a number which is dynamical adjustable because the in vivo half life of σ^{32} is short (in steady state it is 0.7 minutes at 42°C and 15 minutes at 22°C [8, 10]). The life time σ^{32} is known to increase transiently under the HS.

The measurement was on *E.coli* K12 strain grown on A+B medium [12] with a ^3H labelled amino acid added. After the temperature shift we extracted 0.6 ml samples of the culture at subsequent times. Each sample was exposed to radioactive labelled methionine for 30 seconds, after which non radioactive methionine was added in huge excess (10^5 fold). Methionine is an amino acid that the bacteria absorb very rapidly, and then use in protein synthesis. Protein DnaK was separated by 2-dim gel electrophoresis as described by O'Farrell [11], and the amount of synthesis during the 30 seconds of labeled methionine exposure was counted by radioactive activity and normalized first with respect to ^3H count and then with respect to total protein production. This result in an overall accuracy of about 10%. The result is

a count of the differential rate of DnaK production (i.e. the fraction DnaK constitute of total protein synthesis relative to the same fraction before the temperature shift [12]) as function of time after the temperature shift. For the shift $T \rightarrow T + \Delta T$ at time $t = 0$ we thus record:

$$r(T, t) = \frac{\text{Rate of DnaK production at time } t}{\text{Rate of DnaK production at time } t = 0} \quad (1)$$

where the denominator counts steady state production of DnaK at temperature T . In figure 1 we display 3 examples, all associated to temperature changes of absolute magnitude $\Delta T = 7^\circ C$. When changing T from $30^\circ C$ to $37^\circ C$ one observe that r increases to ~ 6 after a time of 0.07 generation. Later the expression rate relaxes to normal level again, reflecting that other processes counteracts the initial response. When reversing the jump, we see the opposite effect, namely a transient decrease in expression rate. Finally we also show a temperature jump at a low temperature, and here we observe the opposite effect, namely that a T increase give a decrease in expression rate. Here a corresponding T decrease in fact gives an increase in expression rate (not shown). Thus the cells response to a positive temperature jump is opposite at low temperature T than it is at high T .

In figure 2 we summarize our findings by plotting for a number of positive shocks $T \rightarrow T + 7^\circ C$ the value of $r = R$ where the deviation from $r = 1$ is largest. This value can be well fitted by the following dependence on temperature T

$$\ln(R(T)) = (\alpha \Delta T) (T - T_0) \quad (2)$$

where $R(T = T_0 = 23^\circ) = 1$ and $\alpha \Delta T = \frac{\ln(R_1/R_2)}{T_1 - T_2} = 0.2 \cdot K^{-1}$ (i.e. $\alpha = 0.03 K^{-2}$).

In order to interpret this result we first assume that the production rate of DnaK is controlled by two factors, a slowly varying factor C that depends on composition of some other molecules in the cell, and an instantaneous chemical reaction constant K . Thus at time t after a shift in temperature the production of DnaK in the cell is:

$$\frac{d[DnaK]}{dt}(t, T \rightarrow T + \Delta T) = C(t, T \rightarrow T + \Delta T) \cdot K(T + \Delta T) \quad (3)$$

where the initial composition of molecules, $C(t = 0, T \rightarrow T + \Delta T)$ equals their equilibrium number at the temperature we changed from, i.e. $= C_{eq}(T)$.

To lowest approximation, where we even ignore feedback from changed DnaK in the cell until DnaK production rate have reached its peak value:

$$R = \frac{K(T + \Delta T)}{K(T)} \quad (4)$$

which implies that

$$\ln(R) = \ln(K(T + \Delta T)) - \ln(K(T)) = \frac{d\ln(K)}{dT} \Delta T \quad (5)$$

Using the linear approximation in Fig. 2

$$\ln(K) \approx \text{const} + \frac{\alpha}{2}(T - T_0)^2 \quad (6)$$

Identifying $K = \exp(-\Delta G/T)$ the effective free energy associated to the reaction is

$$\Delta G \approx G_0 - \frac{\alpha T}{2}(T - T_0)^2 \quad (7)$$

Thus ΔG has a maximum at $T = T_0 = 23^\circ$.

To interpret the fact that HS is connected to a ΔG that have a maximum at $T = T_0 \approx 23^\circ$ we note that many proteins exhibit a maximum stability at T between 10°C and 30°C [13, 14]. Thus $\Delta G = G(\text{folded}) - G(\text{unfolded})$ connected to the folded state of a protein is at a minimum at T_0 . The corresponding maximum of stability is in effect the result of a complicated balance between destabilization from entropy of polymer degrees of freedom at high T , and destabilization due to decreased entropic contribution to hydrophobic stabilization of proteins at low T [14, 15]. One should expect a similar behaviour also for some parts of a protein [15], and thus expect a max binding for hydrophobic protein-protein associations around T_0 . Quantitatively the size of the ΔG change inferred from the measured value of $\alpha = 0.03\text{K}^{-2}$ correspond to a changed G of about $20 \rightarrow 30\text{kT}$ (about 15Kcal/mol), for a temperature shift of about $40\text{-}50^\circ\text{C}$. This matches the change observed for typical single domain proteins [14]. Thus the HS is associated to a ΔG change equivalent to the destabilization of a typical protein.

The above picture still leave us with the puzzle that protein binding and folding stability is at a maximum around T_0 , whereas the effective ΔG we

observe have a minimum there. This can only be reconciled if the interaction we consider is inhibitory. An inhibitory binding that controls the feedback is indeed possible [3]. To summarize our understanding we in figure 3 display the molecular network that we believe is controlling the transient heat shock levels of DnaK in the cell. The key inhibitory control mechanism is the association of DnaK to σ^{32} . DnaK binds to unfolded protein residues [7], and the amount of DnaK· σ^{32} association thereby monitor cellular consequences of a shift in temperature.

Impacts of mutants: We have measured the heat shock in a strain where the σ^{32} gene is located on a high copy number plasmid. In this strain where the synthesis rate for σ^{32} may approach that of DnaK we find a HS that was smaller and also remained positive down to temperature jumps from T well below $T_0 = 23$. According to fig. 3 this reflects a situation where both σ^{32} and DnaK are increased. With increased DnaK level, one may have a situation where DnaK exceeds the amount of unfolded proteins, and free DnaK concentration thus becomes nearly independent of the overall state of proteins in the cell. Also the huge increase in σ^{32} supply may decrease the possibility for the sink to act effectively. Thereby other effects as *e.g.* the temperature dependence of the binding $\sigma^{32} \cdot RNAP$ versus the binding $\sigma^{70} \cdot RNAP$ (i.e. K_{32}/K_{70} from figure 3) may govern a response that otherwise would be masked by a strongly varying inhibition from σ^{32} binding to DnaK.

The reaction network in Figure 3 allow a more careful analysis of the production rate of DnaK:

$$\frac{d[DnaK]}{dt} \propto [RNAP \cdot \sigma^{32}] \approx \frac{[\sigma^{32}]}{1 + g [DnaK]} \quad (8)$$

where the σ^{32} changes when bound to DnaK due to degradation by proteases (the “sink” in figure 3):

$$\frac{d[\sigma_{32}]}{dt} \propto Supply - [DnaK \cdot \sigma^{32}] \approx Supply - \frac{[\sigma^{32}]}{1 + (g [DnaK])^{-1}} \quad (9)$$

Here $g = exp(-\Delta G/T)$ is an effective reaction constant. In the approximation where we ignore free σ^{32} , free σ^{70} and the fraction of DnaK bound by

σ^{32} then:

$$g = \left(\frac{K_{70}[\sigma^{70}]}{K_{32}[RNAP]} \right) \cdot \left(\frac{K_{D32}}{1 + K_{DU}[U_f]} \right) \quad (10)$$

The first term expresses the σ 's competition for RNAP binding whereas the second term expresses the DnaK controlled response. $[U_f]$, which denote unfolded proteins that are not bound to DnaK, decreases with increasing $[DnaK]$.

When moving away from T_0 , i.e. lowering g by increasing $[U_f]$, the rate for DnaK production increases. For an approximately unchanged ‘‘Supply’’ the extremum in production occurs when $d[\sigma^{32}]/dt = 0$ and has a value that approximately is $\propto 1/g$. With the assumption that ‘‘Supply’’ does not have time to change before extreme response is obtained, we identify R with $1/g$ and thereby with the free energy difference ΔG that controls the HS. The early rise in r is reproduced when most σ^{32} are bound to RNAP reflected in the condition $g[DnaK] \ll 1$. This implies a significant increase in σ^{32} lifetime under a positive HS, and implies that the early HS is due to a changed depletion rate of σ^{32} . Later the response is modified, partly by a changed ‘‘Supply’’ and finally by a changed level of the heat shock induced protease HflB that depends on and counteracts the σ^{32} level in the cell.

The largest uncertainty in our analysis is the possibility of a significant time variation in ‘‘Supply’’ and HflB level during the HS. As these will govern the late stages of the heat shock, in particular including its decline, the variation in ‘‘ ΔG ’’ for proteins in the cell may easily be underestimated from using the peak height variation with T . Adding to the uncertainty in what ΔG precisely represents is also the fact that although we only measure DnaK, it can be the complex of the heat shock proteins DnaK, GrpE and DnaJ that sense the state of unfolded proteins in the cell, as removal of any of these display an increased life time of σ^{32} [9, 10]. Such cooperativity may amplify the heat shock.

For the final interpretation of ΔG we stress that it effectively counts the free energy difference between the complex $DnaK \cdot \sigma^{32}$ and that of DnaK being free or being bound to unfolded proteins in the cell. Dependent on the fraction of DnaK relative to unfolded proteins $[U]$ in the cell, i.e. whether $K_{DU}[U_f]$ is larger or smaller than 1, the HS will depend or not depend on the overall folding stability of proteins in the cell. Thus for much more unfolded proteins than DnaK in the cell, the measured ΔG reflect both an increases

of the binding to unfolded residues $K_{DU}[U_f]$ as well as a decrease of the DnaK· σ^{32} binding K_{D32} when moving away from T_0 . Our data does not discriminate between these processes. This discrimination can however be obtained from the data of ref. [16] where it was found that overexpression of DnaK through a σ^{32} dependent pathway in fact repress HS. As DnaK· σ^{32} binding still play a crucial role in this setup, the vanishing HS of [16] support a scenario where too much DnaK imply $K_{DU}[U_f] \ll 1$. Then g in eq. 10 and thereby the σ^{32} response becomes insensitive to the amount of unfolded proteins in the cell.

We conclude that the HS is induced through the changed folding stability of proteins throughout the cell, sensed by a changed need of chaperones. We believe this reflect primarily an increased amount of proteins that are on the way to become folded (nascent proteins), and not an increased denaturation of already folded proteins, because spontaneous denaturation of proteins is extremely unlikely at these temperatures. Thus the deduced sensitivity to the thermodynamic stability of proteins may primarily reflect a correspondingly sensitivity to change in folding times.

We now discuss related proposals of “cellular thermometers” for the HS. McCarthy et al. [17] proposed that the thermometer was a change in autophosphorylation of the DnaK protein. This should cause a temperature dependent activity of this protein. However their data does not indicate that the reversed HS response that we observe at $T < 23\text{C}$ could be caused by such mechanism. Gross [3] made an extensive network of possible chemical feedback mechanisms which connect a rise in σ^{32} level with the folding state of proteins in the cell. It included HS induced through an increased synthesis of σ^{32} , an increased release of σ^{32} from DnaK as well as an increased stability of σ^{32} when DnaK gets bound to unfolded protein residues. Our fig. 3 specifies these possibilities to a minimalistic chemical response including the two latter mechanisms combined, and of these only the option of a changed stability of σ^{32} due to a sink controlled by DnaK· σ^{32} is able to reproduce also the fact that the max HS takes time to develop. In regards to the by Morita [18] proposed increased synthesis of σ^{32} , we note that for high temperatures T , the major mechanism that controls σ^{32} synthesis in fact is a T dependent change in the mRNA structure that leads to an increased translation at increased T [18]. However again our finding of a reversed HS at $T < 23\text{C}$ is not readily explained by such changes in the stability of mRNA structures

below 23C.

In summa, we observed that positive heat shock is induced when T changes away from $T_0 \sim 23^\circ$. We found that the size of the heat shock qualitatively as well as quantitatively follows the thermodynamic stability of proteins with temperature. This suggested that stability related to hot as well as to cold unfolding of proteins is implemented the in HS. We demonstrated that such an implementation was possible in a minimalistic chemical network where the control is through an inhibitory binding of the central heat shock proteins. We finally saw that the temporal behaviour of the HS is reproduced when this inhibitory binding controls the heat shock by exposing σ^{32} to a protease.

References

- [1] S. Lindquist, *Annu. Rev. Biochem.* **55**, 1151 (1986).
- [2] F. C. Neidhardt, R. A. VanBogelen and V. Vaughn, *Annu. Rev. Genet.* **18**, 295 (1984).
- [3] C.A. Gross, Cellular and Molecular Biology, ASM press, 1382 (1996).
- [4] Y. Zhou, N. Kusukawa, J. W. Erickson, C. A. Gross & T. Yura, *Journ. of Bacteriology* **170**, 3640 (1988).
- [5] S. L. Herendeen, R. A. VanBogelen and F. C. Neidhardt, *J. Bacteriol.* **139**, 185 (1979).
- [6] S. Pedersen, P. L. Bloch, S. Reeh and F. C. Neidhardt, *Cell* **14**, 179 (1978).
- [7] A. Gragarov, L. Zeng, X. Zhao, W. Burkholder and M. E. Gottesman *J. Mol. Biol.* **235**, 848 (1994).
- [8] D. B. Straus, W. A. Walter and C. A. Gross, *Nature* **329**, 348 (1987).
- [9] D. B. Straus, W. A. Walter and C. A. Gross, *Genes Dev.* **4**, 2202 (1990).

- [10] K. Tilly, J. Spence and C. Georgopoulos, *Journ. of Bacteriology* **171**, 1585 (1989).
- [11] P.H. O’Farrell, *J. Biol. Chem* **250**, 4007 (1975).
- [12] S. Reeh, S. Pedersen and J. D. Friesen, *Molec. gen. Genet.* **149**, 279 (1976).
- [13] P. L. Privalov, E. I. Tiktopulo, S. Yu. Venyaminov, Yu. V., Griko, G. I. Makhatadze & N. N. Khechinashvili, *J. Mol. Biol.* **205**, 737 (1989).
- [14] G. M. Makhatadze and P. L. Privalov, *Adv. Protein Chem.* **47**, 307 (1995)
- [15] A. Hansen, M. H. Jensen, K. Sneppen and G. Zocchi, *Eur. Phys. J B* **6**, 157 (1998).
- [16] K. Tilly, N. McKittrick, M. Zylicz and C. Georgopoulos, *Cell* **34**, 641 (1983).
- [17] J. S. McCarthy and G. C. Walker, *Proc. Natl. Acad. Sci* **88**, 9513 (1991)
- [18] M. T. Morita, Y. Tanaka, T. S. Kodama, Y. Kyogoku, H. Yanagi and T. Yura, *Genes & Dev.* **13**, 655 (1999).

Figure Captions

- Fig. 1. Heat shock response measures as DnaK rate production change as function of time since a temperature shift. The production is normalized with overall protein production rate, as well as with its initial rate. In all cases we use absolute $\Delta T = 7^{\circ}C$. We stress that the time scale is in units of one bacterial generation measured at the initial temperature. At $T = 37^{\circ}C$ the generation time is 50 min, at $30^{\circ}C$ it is 75 min and at $20^{\circ}C$ it is 4 hours.
- Fig. 2. Induction fold R for positive temperature jumps as function of initial temperature. The straight line correspond to the fit used in eq. 2.

- Fig. 3. Sufficient molecular network for the early heat shock. All dashed lines with arrows in both ends are chemical reactions which may reach equilibrium within a few seconds (they represent the homeostatic response). The full directed arrows represent one way reactions, with the production of DnaK through the $\sigma^{32} \cdot RNAP$ complex being the central one in this work (this step is catalytic, it involves DNA translation etc.) The time and temperature dependence of the early HS is reproduced when most DnaK is bound to unfolded proteins, and when remaining DnaK binds to σ^{32} to facilitate a fast depletion of σ^{32} through degradation by protease HflB.





