Rapid activation of the heat shock transcription factor, HSF1, by hypo-osmotic stress in mammalian cells

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Osmoregulation is important to living organisms for survival in responding to environmental changes of water and ionic strength. We demonstrated here for the first time that exposure of HeLa cells to a hypotonic medium (30% growth medium and 70% water) prominently induced the binding activity of the heat shock transcription factor (HSF). Pretreatment of cells with cycloheximide did not inhibit the induction of HSF-binding activity, indicating that the mechanisms of induction are independent of new protein synthesis. The magnitude of hypo-osmotic stress-induced HSF-binding activity was comparable with that induced by heat shock. The induction, as monitored by gel-mobility-shift assay, occurred within 5 min of hypo-osmotic stress and persisted at least up to 4 h in HeLa cells under the hypotonic conditions. Addition of sorbitol to the hypotonic medium abolished HSF activation. Hypo-osmotic stress-induced HSF binding could also be demonstrated in HeLa cells maintained in simple sorbitol solution by decreasing the sorbitol concentration from 300 mM to 200 mM or less. Competition analysis suggests that the effects of hypo-osmotic stress on HSF-binding activity was specific. Cross-linking experiments and Western-blot analysis demonstrated that hypo-osmotic stress induced trimerization of human heat shock factor 1 (HSF1) in intact HeLa cells, suggesting that trimer formation of HSF1 was responsible for inducing HSF-binding activity in hypo-osmotically stressed cells. However, unlike heat shock response, the activation of HSF by hypo-osmotic stress did not lead to accumulation of hsp70 mRNA in HeLa cells.

INTRODUCTION

Harmful or stressful conditions in the cellular environment can elicit specific cellular responses including the production of specific proteins and the activation of specific genes. One of the most important and extensively studied responses to external stress has been the expression of the heat shock family of proteins [1,2]. The heat shock response and the heat shock proteins (hsps), first discovered in *Drosophila* [3], have since been shown to be elicited by a wide range of noxious stimuli and to be ubiquitous in almost all living organisms studied [4]. Environmental stresses including heat, transition heavy metals, amino acid analogues and oxidants can induce expression of heat shock proteins through an activation of heat shock transcription factor (HSF) [2]. Both the *cis*-acting DNA element and the *trans*-acting protein factor necessary for the heat-induced transcriptional activation of the heat shock genes have been identified and characterized [1,2]. The induction of heat shock genes in response to heat shock and other stresses is mediated by the binding of a transcriptional activator, HSF, to a short, highly conserved upstream response element, termed heat shock *cis*-element (HSE). Heat shock elements (HSEs) are contiguous arrays of variable numbers of 5 bp sequence nGAAn (n stands for less-conserved nucleotides) arranged in alternating orientation [5]. A high-affinity binding of heat shock *trans*-acting factor requires at least two nGAAn units, arranged either head-to-head or tail-to-tail, in the HSE oligonucleotide [6]. The eukaryotic HSF appears to be a multi-zipper protein [7]. In unstressed cells, HSF is present in both the cytoplasm and nucleus in a monomeric form that has no DNA-binding activity. In response to heat shock or other stress, HSF forms a trimer which exhibits high-affinity binding to HSEs present in the heat shock gene promoter [7–9]. The mechanisms by which heat shock or stress is sensed and the pathway that leads to the activation of HSF through trimerization are not clear.

Osmoregulatory processes are important to all living organisms since the maintenance of intracellular osmotic pressure (or chemical potential of metabolites) is of fundamental importance for cell survival [10]. It has also become clear that these processes may have important functions in rapidly proliferating cells because of enhanced solute transport [11]. One approach to investigating these processes is to examine the earlier physiological responses that can be elicited by osmotic shock. For example, it has been shown that fluctuation of medium osmolarity specifically affects expression of certain genes including *OmpF* and *OmpC* in bacteria [12,13]. In eukaryotic cells, it has been shown that hypo-osmotic shock induces ornithine decarboxylase in mammalian cells [14] and polyphosphate hydrolysis in *Neurospora crassa* [15]. Heat shock response occurs almost ubiquitously in living organisms in response to a whole array of environmental insults. It is tempting to speculate that a large fluctuation of environmental osmolarity may elicit heat shock response in cultured cells. In this study we demonstrated that exposure of HeLa cells in culture to a hypo-osmotic stress induced the DNA-binding activity of the HSF through mechanisms independent of new protein synthesis.

MATERIALS AND METHODS

Cell culture and treatment

Tissue-culture supplies were purchased from GIBCO. The human HeLa (A.T.C.C. CCL2) cells were maintained in Dulbecco's modified Eagle's medium supplemented with 6% (v/v) fetal bovine serum in the presence of 50 units/ml of penicillin and

Abbreviations used: HSF, heat shock transcription factor; HSE, heat shock *cis*-element; EGS, ethylene glycol bis(sulphosuccinimidylsuccinate); CTF, CCAAT-binding factor; hsp, heat shock protein; nt, nucleotide(s); DTT, dithiothreitol.

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50 μg/ml of streptomycin at 37 °C. Hypo-osmotic stress was achieved by replenishing the culture medium with an equivalent volume of prewarmed pyrogen-free water [16]. Thus, a hypotonic medium with 70% water dilution indicates that 70% (v/v) of the culture medium was replaced by water. Alternatively, HeLa cells were incubated in isotonic sorbitol solution (0.3 M sorbitol) and hypo-osmotic stress was achieved by reducing the sorbitol concentration. For heat shock, cells were incubated at 42 °C for the times indicated. In experiments to assess the dependence of HSF on new protein synthesis, parallel cultures were treated with cycloheximide (60 μg/ml) for 20 min before hypo-osmotic stress was initiated.

HSE-binding and electrophoretic-mobility-shift assay

Whole-cell extracts were prepared as described previously [17,18]. Briefly, cells were harvested and rapidly frozen at −70 °C. The frozen pellets were suspended in a buffer containing 20 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; pH 7.9), 25% (v/v) glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF and 0.5 mM dithiothreitol (DTT), passed through a 26-gauge needle 20 times, and centrifuged at 100000 ***g*** for 5 min. The supernatants were stored at −70 °C. The protein concentration was determined with BCA Protein Assay Reagent (Pierce). For the binding reaction, 20 μg of whole-cell extracts were mixed in a final volume of 20 μl with a binding buffer containing 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, 5% glycerol and 1 mM DTT in the presence of 0.5 μg of poly(dI-dC) and ~ 250 pg of ^{32}P-labelled oligonucleotides. After 25 min of incubation at room temperature, the resulting complexes were analysed by gel-mobility-shift assay in a low-ionic-strength 4% non-denaturing polyacrylamide gel as previously described [17,19]. The double-stranded synthetic consensus HSE, 5'-GCCTCGAATGTTCGCGAAGT TTCG-3' [17,20] was synthesized in our laboratory by using Gene Assembler Plus DNA synthesizer (Pharmacia) and purified from low-melting-point agarose gels. The oligonucleotide containing the CTF (CCAAT-binding factor)-binding site was purchased from Promega. Both of them were 5' end-labelled with [γ-^{32}P]ATP (ICN) by T4 polynucleotide kinase (Promega) according to the manufacturer's procedures.

Western-blot analysis

Samples (20 μg) of whole-cell extracts were incubated for 20 min at room temperature in the presence of a one-tenth volume of DMSO or ethylene glycol bis(sulphosuccinimidylsuccinate) (EGS, a homobifunctional NHS-ester cross-linker) dissolved in DMSO to a final concentration of 0.6 mM. The reaction was quenched by an addition of glycine to 75 mM. The resultant cell extracts were resolved in a 4–10% gradient SDS/PAGE system, and transferred on to a piece of Immobilon-P transfer membrane (Millipore) using a Mini Trans-Blot Electrophoresis Transfer Cell (Bio-Rad). Non-specific protein-binding sites on the membrane were blocked by incubation with 5% (w/v) non-fat dry milk. The membrane was probed with a 1:5000 dilution of polyclonal anti-HSF1 antibody [7], followed by incubation with anti-(rabbit IgG) antibody conjugated with horseradish peroxidase. The antigen–antibody complex was detected by enhanced chemiluminescence (ECL, Amersham).

RNase protection assay

Total RNA was isolated as described previously [21]. The riboprobe template pRCAT was constructed by inserting a 588 bp *Hin*dIII–*Eco*RI fragment of pGEMCAT [22] into pGEM-3Zf(+) (Promega), and prepared with linearization of pRCAT with *Hin*dIII followed by T7 polymerase-driven *in vitro* transcription using a Riboprobe II Core System kit (Promega), which produced an approx. 600-nt-long riboprobe protecting 150 nt of endogenous hsp70 mRNA. The riboprobe, pT7RNA18S (Ambion), was also *in vitro*- transcribed producing a 109-nt transcript which protects 80 nt of the human ribosomal RNA. RNase protection assays were performed essentially as described previously [22]. Briefly, 4 μg of total RNA was hybridized with both riboprobes simultaneously at 50 °C overnight, digested with RNase T1 and U2, and treated with proteinase K. [^{32}P]-RNA–RNA hybrids were precipitated and resolved on 4% polyacrylamide/8 M urea gels. Gels were fixed in 10% acetic acid, vacuum-dried, and autoradiographed at −70 °C.

RESULTS

Effect of hypo-osmotic stress on HSF binding

Cultured HeLa cells were subjected to hypo-osmotic stress by the water dilution method in which a portion of culture medium was replaced directly by an equivalent amount of prewarmed pyrogen-free water. Thus the percentage of the medium replaced by water would represent a proportional reduction of osmolarity. Figure 1(a) shows that the HSF DNA-binding activity in HeLa cells was prominently induced by hypo-osmotic stress initiated following dilution with 70% water (0.3 × Dulbecco's medium). The degree of osmotic stress, as indicated by the percentage of water dilution required on inducing HSF-binding activity, may vary depending on the cell lines used. For example, 60% water dilution was effective in inducing HSF binding in N2a mouse neuroblastoma cells (results not shown) but not in HeLa cells (Figure 1a, lane 3). Figure 1(b) shows that pretreatment of cells with cycloheximide did not inhibit the hypo-osmotic stress-induced HSF binding, suggesting that new protein synthesis is not a prerequisite for the induction of HSF-binding activity. HSF-binding activity in HeLa cells reached a maximal level at 70% water dilution and remained at this level even under 80% water dilution.

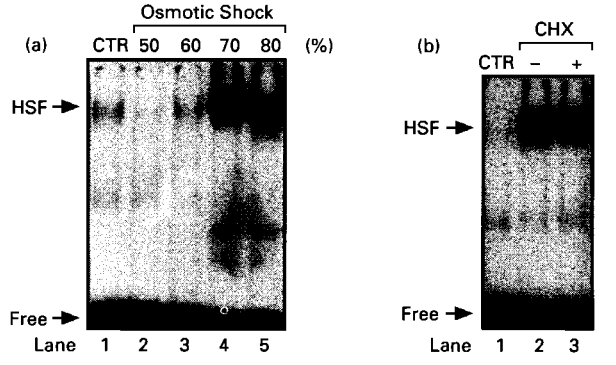

Figure 1 Effects of differential degree of hypo-osmotic stress (a) and cycloheximide treatment (b) on HSF-binding activity

(**a**) Confluent HeLa Cells were incubated in hypotonic Dulbecco's medium at 50 to 80% water dilution for 20 min (lanes 2–5). Cells were then harvested for HSF-binding assay as described in the Materials and methods section. CTR (lane 1) indicates the control culture without hypo-osmotic stress. (**b**) Cells were first treated with cycloheximide (CHX, 60 μg/ml) (lane 3) or without CHX (lane 2) for 20 min before being subjected to hypo-osmotic stress at 70% water dilution for another 20 min. Whole-cell extracts were then prepared for binding and gel-mobility-shift assay using the ^{32}P-labelled HSE as the probe. Lane 1 (CTR), control cells without hypo-osmotic stress.

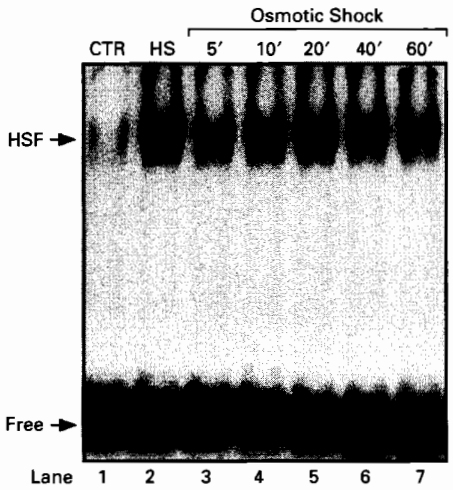

Figure 2 Time course of the induction of the HSF-binding activity by hypo-osmotic stress

HeLa cells at late logarithmic phase were incubated in hypotonic Dulbecco's medium (70% water dilution) for various times at 37 °C as indicated (lanes 3–7). At the end of each incubation time, cells were harvested for cell-extract preparation. HSF-binding and gel-mobility-shift assays were carried out using ^{32}P-labelled HSE as the probe. Lane 1, control culture (CTR); lane 2, culture treated with heat shock (HS) at 42 °C for 20 min. Complex of HSE–HSF (HSF) and excess free probes (Free) are marked.

Time course of the induction of HSF binding by hypo-osmotic stress

Figure 2 shows the time course of the induction of HSF-binding activity in response to hypo-osmotic stress. The HSF-binding activity was prominently induced as early as 5 min and reached a maximal level at about 10 min after 70% water dilution. The magnitude of HSF-binding activity induced by hypo-osmotic stress was comparable with that induced by heat shock at 42 °C (Figure 2, lanes 4–7 versus lane 2). Zimarino and Wu [23] reported that heat shock of intact cells can elicit HSF-binding activity within 30s, reaching a plateau by 5 min. Thus, the time course of the hypo-osmotic stress-induced HSF binding was similar to that induced by heat shock.

Morphology of HeLa cells under hypo-osmotic stress

Figure 3 shows the effect of hypo-osmotic stress on the morphology of HeLa cells. Cells were viable and remained attached to the substratum in hypotonic medium at 60% or 70% water dilution (Figures 3b and 3c). We found that HeLa cells remained viable even incubated in the medium at 80% water dilution for over 12 h (results not shown). However, cells under hypo-osmotic stress exhibited more fibre-like structures at the surface as compared with the control (Figures 3b and 3c versus 3a). The morphology of cells under hypo-osmotic stress at 70% water dilution was similar to that of cells at 60% water dilution (Figures 3c versus 3b). This is interesting since HSF-binding activity of HeLa cells at 60% water dilution was much lower than that in cells at 70% water dilution. The results suggest that the effect of hypo-osmotic stress on morphology or volume may not be directly related to the induction of HSF-binding activity.

Specificity of the effect of hypo-osmotic stress on HSF binding

To demonstrate the specificity of HSF binding, we compared the effect of both heat shock and hypo-osmotic stress on DNA-binding activity using HSE and CCAAT-binding factor (CTF) as the probe. The CTF was chosen because it is one of the *trans*-acting factors that bind to specific *cis*-acting elements on the heat shock promoter [18]. Figure 4 shows that both heat and water loading induced HSE binding but had no effect on CTF binding, suggesting that the effect of hypo-osmotic stress on HSF binding is rather specific. However, we cannot rule out the possibility that hypo-osmotic stress may specifically induce or activate other *trans*-acting factors.

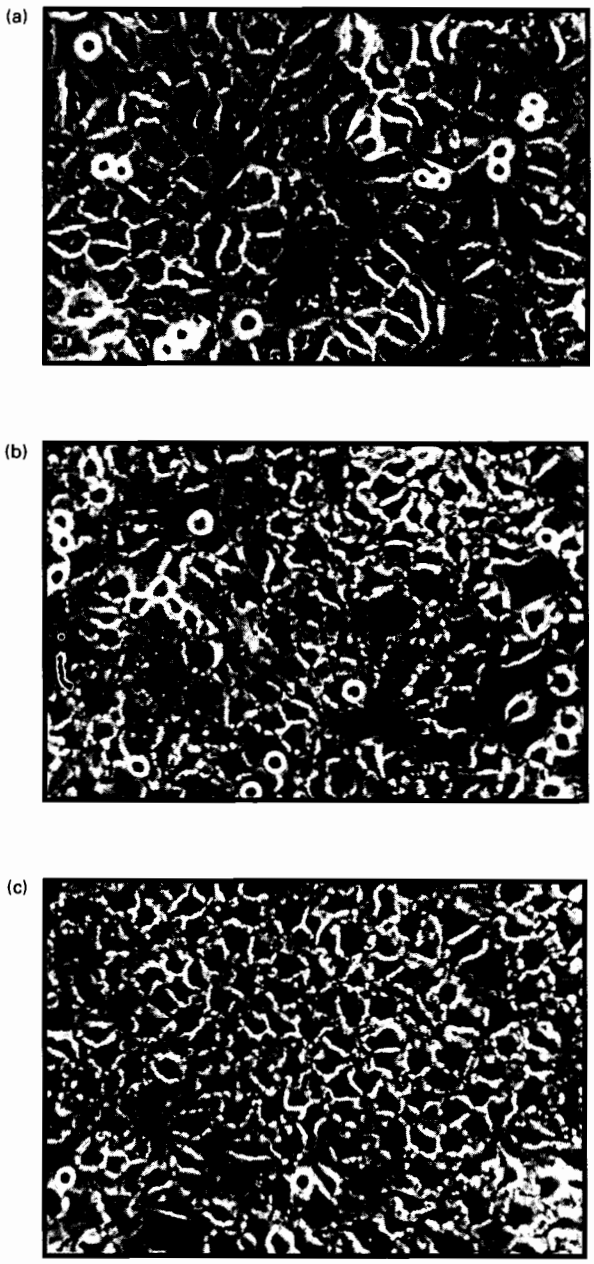

Figure 3 Phase-contrast micrographs of HeLa cells

(**a**) Cells were incubated in Earle's balanced salt solution (EBSS); (**b**) cells were incubated for 60 min in the hypotonic EBSS medium at 60% water dilution; (**c**) cells were incubated for 60 min in the hypotonic EBSS medium at 70% water dilution.

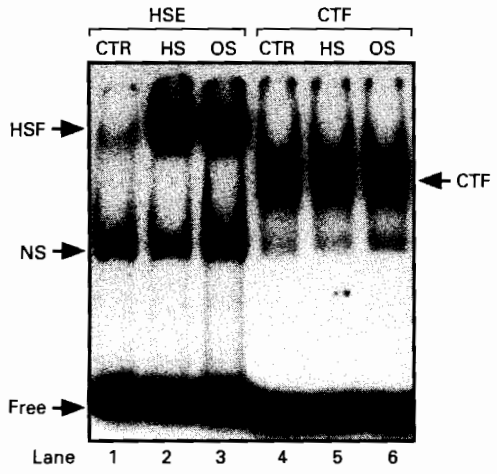

Figure 4 Specificity of hypo-osmotic stress on the induction of HSF-binding activity

Whole-cell extracts prepared from control (lanes 1 and 4), heat-shocked (lanes 2 and 5), and hypo-osmotically stressed cells (lanes 3 and 6) were subjected to a gel-mobility-shift assay using ^{32}P-labelled HSE (lanes 1–3) or ^{32}P-labelled CTF (lanes 4–6) as the probe. Complexes of HSE–HSF (HSF), CCAAT–CTF (CTF), and non-specific DNA-binding (NS), and excess free probes (Free) are indicated.

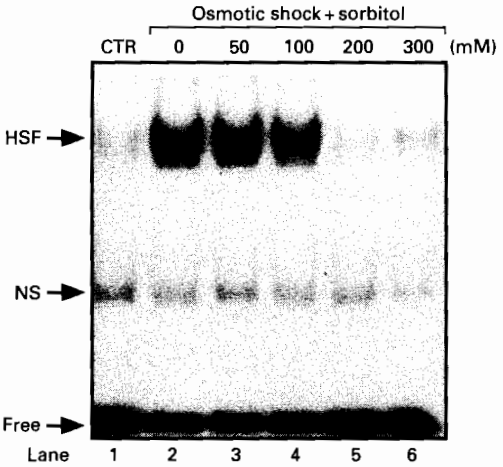

Figure 5 Effects of sorbitol on the HSF-binding activity induced by water dilution

Cells were incubated for 20 min in hypotonic medium (70% water dilution) in the presence of various amounts of sorbitol as indicated (lanes 2–6). Whole-cell extracts were prepared from these cells for binding and gel-mobility-shift assay using ^{32}P-labelled HSE as the probe. Lane 1, control cells without treatment. The positions of complexes of HSE–HSF (HSF), non-specific DNA-binding (NS), and excess free probes (Free) are marked.

Effect of sorbitol on HSF binding induced by water dilution

Although water dilution has been frequently employed as a means to produce hypo-osmotic stress [16], the procedure causes changes not only in osmolarity but also ionic composition of the medium. To delineate the contributing cause for the induction of HSF-binding activity, we examined the effects of sorbitol on HSF binding induced by water dilution. Figure 5 shows that an addition of sorbitol at 200 mM or more to the hypotonic medium at 70 % water dilution could block the induction of HSF-binding

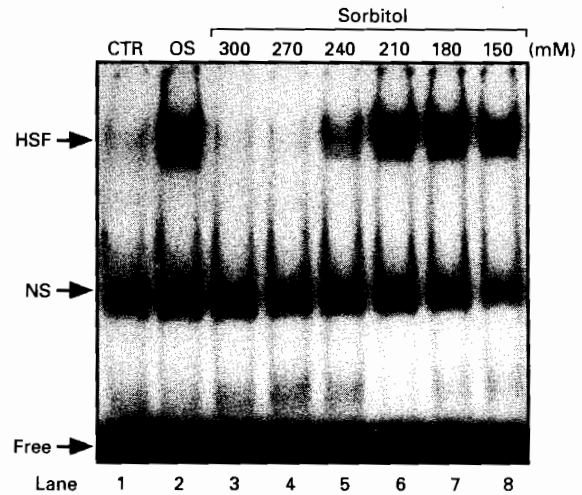

Figure 6 Induction of HSF-binding activity in HeLa cells maintained in simple sorbitol solution

Confluent cells were washed first with isotonic sorbitol solution (300 mM) once and then incubated in pure sorbitol solution at indicated concentrations (lanes 3–8) for 20 min at 37 °C. Lane 1, control culture (CTR); lane 2, culture incubated in hypotonic Dulbecco's medium (70% water dilution) for 20 min (OS). Whole-cell extracts were prepared from these cells and subjected to binding and gel-mobility-shift assay using ^{32}P-labelled HSE as the probe. The positions of complexes of HSE–HSF (HSF), non-specific DNA-binding (NS), and excess free probes (Free) are marked.

activity (Figure 5, lanes 5 and 6 versus lanes 2–4), suggesting that change in osmolarity rather than ionic composition is the main cause for the induction of HSF-binding activity.

Induction of HSF binding in HeLa cells maintained in simple sorbitol solution

The observation that sorbitol at 200 mM or higher can inhibit HSF binding in HeLa cells induced by water loading prompted us to examine the possibility of studying the effects of hypo-osmotic stress on HSF binding in cells maintained in simple sorbitol solution. Figure 6 shows the HSF-binding activities in HeLa cells following an incubation in sorbitol solution at different concentrations for 20 min. The results demonstrated a close relationship between osmolarity of the solution and the HSF-binding activity. The maximal HSF-binding activity, comparable with that induced by 70 % water dilution (Figure 6, lanes 6–8 versus lane 2), occurred at a sorbitol concentration of 210 mM or less (Figure 6, lanes 6–8).

Mechanism of the induction of HSF binding

Two members of the HSF family, HSF1 and HSF2, are known to function as transcriptional activators for heat shock genes [24,25]. Previous studies have identified HSF1 as the mediator of heat shock gene transcription in response to stress conditions, whereas HSF2-binding activity is induced in erythroleukaemia cells by haemin treatment [26]. To examine whether trimerization is involved in the HSF binding induced by hypo-osmotic stress, we carried out Western-blot analysis in control and both heat shock- and hypo-osmotic stress-treated cells using antiserum against human HSF1. Whole-cell extracts prepared from control and treated cells were first incubated in the absence or in the presence of cross-linking reagent EGS and then resolved on SDS/polyacrylamide gel and analysed by antiserum made against

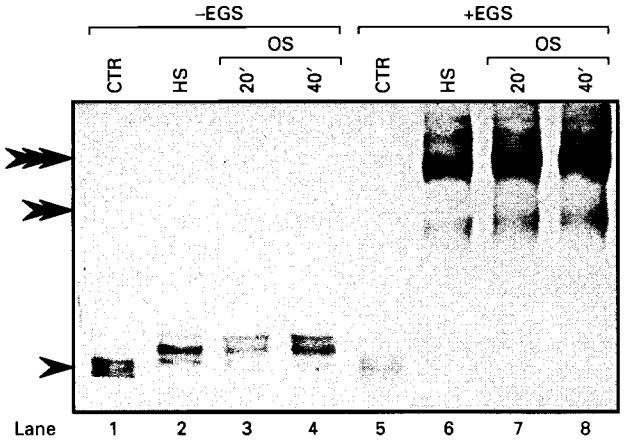

Figure 7 Western-blot analysis of the HSF in HeLa cells during hypo-osmotic stress

HeLa cells were incubated under control (CTR, lanes 1 and 5), heat shocked (HS, 42 °C for 20 min, lanes 2 and 6) or hypo-osmotically stressed (OS, 70% water dilution) conditions at 37 °C for 20 min (lanes 3 and 7) or 40 min (lanes 4 and 8). Whole-cell extracts were prepared from these cells as described in the Materials and methods section. The cell extracts were then incubated in the absence (lanes 1–4) or in the presence (lanes 5–8) of cross-linker EGS for 20 min at 25 °C. The reaction mixture was subjected to SDS/PAGE and Western-blot analysis using polyclonal anti-(human HSF1) antibody. Single, double and triple arrowheads indicate respectively, the positions of mono-, di-, or tri-meric forms of HSF1 on SDS/polyacrylamide gel.

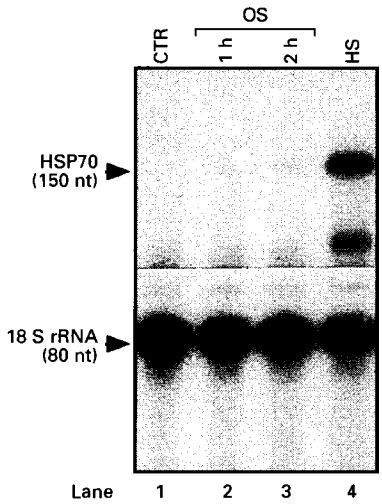

Figure 8 RNase protection analysis of the steady-state level of hsp70 mRNA

HeLa cells were incubated under control (CTR, lane 1), hypo-osmotically stressed (OS, lanes 2 and 3) for 1 or 2 h, or heat shocked (HS, lane 4) conditions for 2 h. Total RNA was isolated from these cells and analysed by RNase protection assay as described in the Materials and methods section. Protected 150 nt of the hsp70 mRNA and 80 nt of 18 S rRNA are marked.

HSF1. The mobility of trimerized HSF1 should be much slower after cross-linking with EGS on SDS/polyacrylamide gel. Figure 7 shows that without EGS, the mobility of HSF1 in cells treated by heat shock or hypo-osmotic stress was at a monomeric position, similar to that of the control (Figure 7, lanes 2–4 versus lane 1). In the presence of EGS, however, the HSF1 in the control remained at the monomeric position (Figure 7, lane 5), but the cross-linked HSF1 in cells treated by either heat or hypo-osmotic stress moved to the trimeric position (Figure 7, lanes 6–8), suggesting that trimerization of HSF1 is the activation mechanism in HeLa cells during hypo-osmotic stress. It is also of interest to note that the mobility of monomeric HSF1 in both heat-shocked cells (Figure 7, lane 2) and hypo-osmotically stressed cells (Figure 7, lanes 3 and 4) was slightly different from that in the control cells (Figure 7, lane 1), suggesting that HSF1 in heat-treated and in hypo-osmotic stress-treated cells may have been modified post-translationally (e.g. phosphorylation) in a similar fashion.

Effect of hypo-osmotic stress on the accumulation of hsp70 mRNA

Finally, we examined whether the activation of HSF and the enhanced HSF-binding activity in hypo-osmotic-stressed cells could lead to an accumulation of hsp70 mRNA. Figure 8 shows that, in contrast to heat shock response, no appreciable hsp70 mRNA was accumulated during hypo-osmotic stress under our experimental conditions.

DISCUSSION

There are three general classes of conditions known to result in the elevated expression of stress proteins: (i) environmental stress such as heat shock, amino acid analogues, transition heavy metals, and inhibitors of energy metabolism; (ii) pathological stages; and (iii) non-stressful conditions [2]. The common feature of these conditions is that they all induce the activation of HSF and that the activation of HSF is the initial and key step necessary for the expression of heat shock genes [1,2]. All living organisms are embedded in an aqueous environment and are sensitive to changes in the osmolarity of the medium they are in. It is therefore necessary for living organisms to adopt effective strategies to cope with osmotic stress. Many organisms synthesize or sequester what are termed 'non-perturbing osmolytes' which have favourable effects on macromolecular species such as the stabilization of enzymes as well as a regulatory control of cell volume [27]. It has also been reported that changes in the osmotic pressure of media can induce changes in gene expression in mammalian cells [28], in yeast [29], as well as in bacteria [30]. In light of the almost ubiquitous heat shock response to various forms of stress it is somewhat intriguing that the effect of hypo-osmotic stress on heat shock response has not been previously investigated.

Our present study clearly demonstrated that hypo-osmotic stress could induce high binding activity to HSE in mammalian cells (Figures 1, 2 and 6) through activation of HSF. The hypo-osmotic stress-induced HSF activation shares a number of similarities to that induced by heat shock, including: (i) both are independent of protein synthesis (Figure 1); (ii) both are rapid (Figure 2); and (iii) both activate HSF through trimerization (Figure 7). In addition, the magnitude of induced HSF-binding activity in both cases was comparable. However, unlike heat shock, which causes a gradual activation of HSF as a function of temperature increase [31], the activation of HSF by hypo-osmotic stress occurred rather abruptly over a narrow range of water dilution, from 60% to 70% (Figure 1). It seems that there exists a threshold of tolerance in the cell to hypo-osmotic stress and that the activation of HSF through its own trimerization occurs only if this threshold is crossed over. Since the signal(s) that leads to HSF activation during heat shock or hypo-osmotic stress has yet to be identified, it remains to be seen whether hypo-osmotic stress and heat shock share identical or similar intracellular signals. In this regard, however, it is of interest to note that direct activation of HSF can be achieved in a HeLa cell-free system by

conditions that affect protein conformation, including increasing concentrations of protons, urea, or non-ionic detergents [31]. It is conceivable that many environmental stresses, including hypo-osmotic stress, may reproduce similar biochemical conditions in the intracellular milieu that can cause HSF activation.

One of the major challenges in studying the regulation of heat shock response is the identification of the intracellular signals that lead to the activation of HSF. The rapid induction of HSF binding in hypo-osmotically stressed cells provides a good model system with a well-defined time frame for studying the signal transduction pathways involved in HSF activation and HSE binding. Such studies may also shed light on the mechanisms involved in heat shock response. The use of sorbitol solution, instead of culture medium, to elicit a hypo-osmotic stress response (Figure 6) eliminates the potential complication due to the nutrient's dilution or changes in ionic composition.

Previous studies have strongly suggested that the activation of HSF1 by heat or other forms of stress is mediated by trimer formation [7,9]. Hypo-osmotic stress clearly induced trimerization of HSF1 in HeLa cells to a similar degree as that in heat-shocked cells (Figure 7). It has been proposed that the C-terminal zipper in HSF1 may suppress trimer formation by the N-terminal zipper elements by means of intramolecular coiled-coil interactions that are sensitive to heat shock [7]. Further studies of the protein chemistry of HSF1 may yield the clue to how this protein can sense a whole array of different stresses by monomer–trimer transition.

Hypo-osmotic stress in prokaryotic and eukaryotic cells can induce many specific physiological responses, including induction of gene expressions such as OmpF, OmpC, and proU in bacteria [12,13,32] and ornithine decarboxylase in mammalian cells [14,33]. Most cells, both eukaryotic and prokaryotic, also respond to osmotic stress by accumulating high intracellular concentrations of compatible solutes (e.g. glycine betaine) to balance external molarity and restore turgor [13]. In response to hypo-osmotic stress, many cell types also express regulatory volume decrease (RVD) through changes in specific ionic fluxes [16]. The rapid activation of HSF in response to hypo-osmotic stress raises an interesting possibility that HSF activation may be involved in some, if not all, of these osmoregulatory responses.

Unlike heat shock, we did not observe a concomitant accumulation of hsp70 mRNA during hypo-osmotic stress of HeLa cells (Figure 8). A pulse-labelling experiment carried out in hypo-osmotically stressed cells did not reveal accumulation of hsp70 and other heat shock proteins (results not shown). Since it has been reported that hypo-osmotic stress can induce accumulation of β-actin and c-*jun* mRNA [28], the lack of hsp70 mRNA accumulation in hypo-osmotically stressed cells may not be due to a total shut down of mRNA synthesis. Thus induction of high levels of HSF binding may not be sufficient for the induction of hsp70 mRNA synthesis. In this regard, it can be noted that both arsenite [34] and salicylate [35] induce high levels of HSF binding but do not elicit the transcription of heat shock genes. Nevertheless, the cause and the physiological significance of this apparent decoupling of HSF binding and transcription of heat shock genes need to be further investigated.

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