Heat Shock Factor 1 Mediates Hemin-induced hsp70 Gene Transcription in K562 Erythroleukemia Cells*

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Transcriptional induction of the hsp70 gene is mediated by heat shock factor 1 (HSF1) rapidly activated upon heat and other stresses. HSF2 has been thought to be responsible for accumulation of HSP70 during hemin-induced differentiation of human K562 erythroleukemia cells because of accompanying acquisition of HSF2 DNA binding activity. However, there has not been any direct evidence for such a functional role of HSF2. The purpose of this study is to clarify the roles of HSF1 and HSF2 in HSP70 induction in hemin-treated K562 cells. We show here that a chimeric polypeptide of HSF2 and GAL4 DNA binding domain (GAL4-BD–HSF2) was unable to induce a GAL4 binding site-containing luciferase reporter gene in response to hemin and that exogenously overproduced HSF2 also failed to increase expression of a heat shock element-containing reporter. On the contrary, expression of a GAL4-BD–HSF1 chimeric protein responded to hemin treatment as well as to heat shock, and transiently overexpressed HSF1 caused hemin-responsive induction of the reporter gene in a dose-dependent manner. These results indicate that HSF1, rather than HSF2, primarily mediates the hemin-induced transcription of the hsp70 gene.

Cells synthesize increased levels of HSP70 upon various environmental stresses such as elevated temperatures, heavy metals, and amino acid analogs to cope with the deleterious circumstances (1). The expression of HSP70 is also increased in mouse embryonal carcinoma cells (2), at the two-cell stage of embryogenesis (3), during spermatogenesis (4), and during S-phase of the cell cycle (5). Heat shock factors (HSFs)1 are responsible for transcription of the hsp70 gene upon these stressful or other physiological conditions, and multiple HSF genes have been isolated in chicken (HSF1, HSF2, and HSF3), mouse (HSF1 and HSF2), and human (HSF1, HSF2, and HSF4) (6–10). Whereas HSF1 and HSF3 are rapidly activated by various stresses and stimulate transcription of the hsp70 gene (11–14), it remained to be clarified which of the HSFs is responsible for the hsp70 expression under non-stressed conditions.

Human K562 erythroleukemia cells, established from a patient with chronic myelogenous leukemia in blast crisis (15), are multipotent stem cells and differentiate to erythroid cells by treatment with hemin or sodium butyrate or hydroxyurea (16–18). During hemin-mediated erythroid maturation of K562 cells, accumulation of HSP70 (19) and elevated levels of activated HSF2 (20, 21) have been observed. Increased expression of HSP70 and constitutive DNA binding of HSF2 have also been reported in mouse spermatogenic cell types (22), embryonal carcinoma cells (23), and postimplantation embryos from E8.5 to E15.5 (24). However, whether HSF2 mediates the transcriptional activation of the hsp70 gene under these non-stress conditions has been controversial for lack of direct evidence.

We recently analyzed the functional domains of human HSF2 in K562 cells using chimeric constructs fused to the GAL4 DNA binding domain (GAL4-BD) and identified the transactivation domains and negative regulatory motifs (25). Although chimeric constructs of HSF1 fused to GAL4-BD had been shown to respond to heat shock by activating transcription of the heat shock element (HSE)-containing reporter genes (26–28), all of the GAL4-BD–HSF2 chimeras failed to respond to hemin treatment. Such an unexpected behavior of HSF2 prompted us to investigate which HSF mediates the increased expression of HSP70 in hemin-treated K562 cells.

Here, we report that, in K562 cells (ATCC CCL243), HSF1 but not HSF2 responds to hemin to activate transcription of the hsp70 gene. GAL4-BD–HSF1 chimeric protein stimulated expression of a luciferase reporter gene containing GAL4 binding sites upon not only heat shock but also hemin treatment. Transiently overexpressed HSF1 increased expression of an HSE-containing reporter in response to hemin in a dose-dependent manner, but transient expression of HSF2 did not show any significant effects on the hemin response. Moreover, HSF2-overproducing stable transformants lacked hemin-responsive expression of HSP70 despite constitutive HSF2 DNA binding. These results suggest that HSF1 primarily mediates transcription of the hsp70 gene during hemin-induced differentiation of K562 cells.

EXPERIMENTAL PROCEDURES

Plasmid Constructions—The fusion gene of HSF2N96 or HSF1N101 with GAL4-BD was inserted into a mammalian expression vector pCAGGS (29) as described previously (25). To construct HSF1–2N101, residues 215 to 378 of HSF1 were replaced with residues 204 to 354 of HSF2 by polymerase chain reaction. In HSF2–1N96, residues 210 to 321 of HSF1 were substituted for residues 199 to 327 of HSF2. HSF2 and HSF1 expression plasmids, pCAHSF2 and pCAHSF1, were constructed by ligation into pCAGGS (29) of the DNA fragments encoding human HSF1 and HSF2 prepared from pHSP2–1 (provided by R. E. Kingston) and pHSF1 (30), respectively. pCKHSF2 was constructed by insertion of the HSF2 fragment into pCXN2 (29) to generate HSF2-overproducing stable cell lines. The firefly luciferase reporter plasmid pGLHSE3 was constructed by insertion into pGL3-promoter vector (Promega) of three tandem repeats of an HSE-containing synthetic DNA, whose sequence was identical to that of the probe used in gel shift analysis (see below). GAL4 binding site-containing luciferase reporter plasmid pGL4E5 was described previously (30). Renilla luciferase expression plasmid pRLSV40 was purchased from Promega.

Transient Transfection and Luciferase Assay—K562 cells (ATCC CCL243) were cultured in RPMI 1640 medium containing 10% fetal...
bovine serum in 5% CO₂ at 37 °C. Transient transfection of K562 cells was done by electroporation. 5 × 10⁵ cells were washed twice with phosphate-buffered saline (PBS) and suspended in 0.4 ml of PBS containing 30 μg of plasmids. After incubating for 10 min at room temperature, the cells were exposed to an electric pulse (300 V/cm, 500 microfarads) by using Gene Pulser (Bio-Rad). The electroporated cells were left at room temperature for 10 min and then incubated in RPMI 1640 medium containing 10% fetal bovine serum in 5% CO₂ at 37 °C for 48 h. Cells were harvested, washed twice with PBS, divided into two aliquots for luciferase assay and for preparation of cell extracts, and stored at −80 °C. Luciferase assays were performed by using Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s instruction.

**Generation of Stable Lines**—K562 cells were transfected with 20 μg of linearized pCXHSF2 DNA as described above. Cells were cultured for 2 days at 37 °C, and neomycin-resistant cells were selected in medium containing 300 μg/ml of G418 for 4 days. Thereafter, the culture was diluted, and the selection was continued further for 2 weeks to obtain single cell-derived clones.

**Whole Cell Extracts Preparation**—Cell pellets were frozen at −80 °C and suspended in 20 mM HEPES containing 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM dithiothreitol, 25% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, and 10 μg/ml aprotinin. After addition of NaCl to a final concentration of 0.42 M, the lysates were set on ice for 5 min and centrifuged for 15 min at 100,000 × g at 4 °C. The supernatants were used as whole cell extracts. Protein concentrations were determined by a protein assay kit (Bio-Rad) using bovine serum albumin as a standard.

**Northern Analysis**—Total RNA from K562 cells was prepared by using Isogen (Nippon Gene) and separated on a 1% agarose gel containing formaldehyde. The RNA was transferred onto a nylon membrane (DuPont NEN), hybridized with ³²P-labeled human hsp70 genomic DNA (SPD-925) (StressGen), grp78 genomic DNA (nucleotides +3 to +593 prepared from HeLa genomic DNA by polymerase chain reaction), or glyceraldehyde-3-phosphate dehydrogenase cDNA (CLONTECH), washed in 0.2 × SSPE, 0.1% SDS at 42 °C, followed by autoradiography and quantification with a BioImaging analyzer BAS2000 (Fuji Film).

**Gel Mobility Shift Assay**—Whole cell extracts (10 μg of protein) were mixed with 5 fmol of double-stranded ³²P-labeled oligonucleotide (5′-GATCCGGAGGTGAAACCCCTTGAATATTCTCGACCTGGCAGATC–3′) containing HSE of the human hsp70 promoter (−120 to −85) (31) in 25 μl of reaction buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol) containing 1 μg of poly(dI-dC) (Amersham Pharmacia Biotech). The mixture was incubated for 20 min at room temperature, and 15 μl was loaded onto a 4% polyacrylamide gel in 0.15 × TAE. After electrophoresis at 250 V for 2 h at 4 °C, the gel was dried on DE81 paper and exposed to x-ray film. Gel shift assay using a probe containing a single GAL4 binding site was described previously (25).

**Western Blotting**—Polyclonal rabbit antibodies raised against chicken HSF2 (αHSF2a and αHSF2b) or chicken HSF1 (αHSF1) (kindly provided by A. Nakai) or human HSF1 (PA3–017) (Affinity BioReagents) were used. Whole cell extracts (20 μg of protein) were subjected to SDS-polyacrylamide gel electrophoresis (10% polyacrylamide), and proteins were electroblotted onto nitrocellulose membranes (Amersham). The filters were blocked with 5% skim milk in PBS containing 0.1% Tween 20 (PBST) for 1 h at room temperature and incubated with primary antibodies (1:2000 dilution) in PBST containing 2% skim milk for 1 h at room temperature. After washing with PBST, the membranes were incubated with horseradish peroxidase-conjugated goat F(ab')₂ anti-rabbit antibody (1:2000 dilution) (BioSource) for 1 h at room temperature. Following washing with PBST again, signals were detected using ECL reagent (Amersham) and quantified with Intelligent Quantifier (Bio Image).

**RESULTS**

**Hemin-induced hsp70 Transcription and HSF DNA Binding**—Acquisition of HSE binding activity of HSF2 and transcription induction of the hsp70 gene were examined in hemin-treated K562 cells by Northern blotting, Western blotting, and gel mobility shift analysis (Fig. 1). mRNA levels of hsp70 increased 1.8-, 4.5-, and 10.3-fold by 15, 30, and 60 μM hemin treatment for 12 h, respectively, and 7.8-fold by heat shock (42 °C for 1 h) (Fig. 1A, lanes 1–5). When K562 cells were treated with 60 μM hemin, 4.0-fold accumulation of hsp70 mRNA was observed at 6 h, and the maximum level (8.5-fold) was attained at 12 h (lanes 6, 8, and 9). The transcripts of grp78 also increased 4.5–6.6-fold (lanes 1, 4, 6, and 9) with 60 μM hemin at 12 h. The maximum level of HSE binding was observed in the cells treated with 60 μM hemin for 12 h (Fig. 1C, lanes 4 and 9), and the induction of the HSE binding activities was correlated well with the increase of hsp70 mRNA. These results were consistent with the observations reported previously (20, 21).

It has been reported that the amount of HSF2 is enhanced during hemin treatment (32, 33). By Western blot analysis, we also confirmed increase of the HSF2 level in K562 cells upon treatment with 30 and 60 μM hemin for 12 h (Fig. 1B). Moreover, increase of slower migrating forms of HSF1 was observed in the hemin-treated cells; the extent of mobility change seemed to depend on the hemin concentration (Fig. 1B, lanes 1–4), as has been observed with HSF1 hyperphosphorylated...
upon heat shock (lane 5 and Ref. 11). The upper band was observed between 3 and 24 h of hemin treatment (lanes 7–10). Because hyperphosphorylation has been shown to be required to render HSF1 transcriptionally competent (34, 35), these results raised the possibility that hemin induces HSF1 hyperphosphorylation leading to hsp70 transcription.

**Hemin-induced HSF1 DNA Binding Activity**—To examine which HSF is involved in the hemin-induced HSF binding complexes, we treated cell extracts with specific antisera raised against HSF1 or HSF2 and performed gel mobility shift analysis. As shown in Fig. 2, only a small part of the HSE binding complexes induced by 30 or 60 μM hemin were recognized by HSF2 antiserum (lanes 2–4 and 9–11) under the conditions that caused complete supershift for overexpressed HSF2–HSE complex (lanes 23–25) but not for heat shock-induced complex (lanes 16–18). On the other hand, almost all complexes observed in the hemin-treated cell extract were recognized by HSF1 antiserum (lanes 5–7 and 12–14) as well as those observed in the heat-shocked cell extract (lanes 19–21), although the supershift seemed to be somewhat limited as compared with that observed in the heat-shocked cell extract (compare lanes 13 and 20). These results clearly indicated that the hemin-induced HSE binding complexes consist of both HSF1 and HSF2, the majority being HSF1.

**Heat or Hemin Responsiveness of GAL4-BD–HSF1 and GAL4-BD–HSF2 Constructs**—We then examined which HSF is involved in the transcriptional activation induced in hemin-treated K562 cells by luciferase reporter assay. Constructs HSF2N96 and HSF1N101 have almost full-length of the coding regions of HSF2 and HSF1, respectively, except for their DNA binding domains that were replaced by GAL4-BD (30). Constructs similar to HSF1N101 had been shown to respond to heat shock and activate transcription of appropriate reporter genes (26, 27, 36). The heat shock regulatory domain of HSF1 was substituted for the corresponding region of HSF2 and vice versa, to yield chimeric constructs (Fig. 3A). DNA binding activity of these constructs in K562 cells was confirmed by gel mobility shift assay using a single GAL4 binding site as a probe (Fig. 3B).

The transactivation activity of construct HSF2N96 was entirely repressed under normal conditions and was not induced significantly by exposure to hemin or heat shock (Fig. 3A, lane 2). The chimeric construct HSF2+1N96 also showed little response to hemin or heat shock (lane 3). It is noteworthy that HSF2+1N96 containing the heat shock regulatory domain of HSF1, which had been shown to be able to confer heat responsiveness to a heterologous transactivation domain (28), failed to activate transcription upon heat shock. As both constructs contain the negative regulatory 18-amino acid segment that suppresses the C-terminal transactivation domain of HSF2 (25), these results suggest that the negative segment of HSF2 is fully functional in hemin- or heat shock-treated K562 cells regardless of the regulatory domain of HSF1. On the other hand, construct HSF1N101 elevated the reporter expression 3- and 10-fold in response to hemin and heat shock, respectively (lane 4). Furthermore, construct HSF1+2N101 lacking the heat shock regulatory domain was deregulated and resulted in constitutive activation of the reporter (lane 5). Thus, HSF2 did not show any distinct response to hemin and heat shock, whereas HSF1 responded well to both stimuli, at least under the conditions that GAL4-BD was used as a DNA binding domain.

**Effects of Transiently Overexpressed HSF1 and HSF2**—To further address the involvement of HSF1 in hemin-induced transcription of the hsp70 gene, exogenous HSF1 or HSF2 was transiently expressed in K562 cells, and expression of the cotransferred HSE-containing luciferase gene was examined. As shown in Fig. 4A, the reporter response of the HSF2-transfected cells was quite similar to that of the mock-transfected cells, which represents the response mediated by endogenous HSFs. In contrast, transiently expressed HSF1 markedly enhanced the reporter response not only to heat shock but to hemin treatment (30 and 60 μM) as well.

When whole cell extracts prepared from the above transfected cells were examined by gel mobility shift analysis, the binding of transiently expressed HSF1 was markedly increased by treatment with either hemin or heat shock (Fig. 4B, lanes 4–9). The transactivation activity of construct HSF2N96 was entirely repressed under normal conditions and was not induced significantly by exposure to hemin or heat shock (Fig. 3A, lane 2). The chimeric construct HSF2+1N96 also showed little response to hemin or heat shock (lane 3). It is noteworthy that HSF2+1N96 containing the heat shock regulatory domain of HSF1, which had been shown to be able to confer heat responsiveness to a heterologous transactivation domain (28), failed to activate transcription upon heat shock. As both constructs contain the negative regulatory 18-amino acid segment that suppresses the C-terminal transactivation domain of HSF2 (25), these results suggest that the negative segment of HSF2 is fully functional in hemin- or heat shock-treated K562 cells regardless of the regulatory domain of HSF1. On the other hand, construct HSF1N101 elevated the reporter expression 3- and 10-fold in response to hemin and heat shock, respectively (lane 4). Furthermore, construct HSF1+2N101 lacking the heat shock regulatory domain was deregulated and resulted in constitutive activation of the reporter (lane 5). Thus, HSF2 did not show any distinct response to hemin and heat shock, whereas HSF1 responded well to both stimuli, at least under the conditions that GAL4-BD was used as a DNA binding domain.

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FIG. 3. Transcriptional activation by transiently expressed GAL4-BD–HSF2 and GAL4-BD–HSF1 constructs. K562 cells were transfected with 5 µg of expression plasmid of GAL4-BD or GAL4-BD–HSFs (lanes 1–5), 2 µg of pGL4E5, 0.5 µg of pRLSV40, and 22.5 µg of pBluescript II. After 24 h, transfected cells were treated with 60 µM hemin for 24 h. Alternatively, at 40 h after transfection, cells were heat shocked for 1 h at 42 °C and incubated further for 6 h at 37 °C. Cells were then harvested and subjected to luciferase assays and to gel mobility shift assays. A, the firefly luciferase activity of each transfection was normalized with Renilla luciferase activity and presented as the mean ± SD (bars) from four independent experiments. Expression plasmids of GAL4-BD–HSFs are schematically shown on the left. HR-A/B, hydrophobic repeats; HR-C, C-terminal hydrophobic repeat; AD, C-terminal activation domain; NS, negative regulatory segment of HSF2 (residues 428–445); RD, heat shock regulatory domain of HSF1. The regions derived from HSF1 are shaded. B, whole cell extracts prepared from each transfection were mixed with the 32P-labeled probe containing a GAL4 binding site and were loaded on a native 4% polyacrylamide gel.

FIG. 4. Effects of transiently expressed HSF2 or HSF1 on response to hemin or heat shock. Transient transfection of pCAGGS (Mock), pCAHSF2 (HSF2), or pCAHSF1 (HSF1) was done as described in the legend to Fig. 3 except for using pGLHSE3 instead of pGL4E5. After 24 h, cells were treated with hemin at 15, 30, or 60 µM for 24 h or heat shocked for 1 h at 42 °C. Hemin-treated cells were harvested immediately and subjected to gel shift analysis, Western blotting, and luciferase assay. Heat-shocked cells were incubated further for 6 h at 37 °C prior to luciferase assay. A, luciferase assay was performed as described in Fig. 3. B, upper panel, gel shift analysis was done using the 32P-labeled HSE probe; lower panel, expression of HSF2 and HSF1 in transiently transfected cells was detected by Western blotting using anti-HSF2 (αHSF2α) and anti-HSF1 (PA3–017) serum, respectively.
versus 6 and 7 versus 9). No such increase in binding was detected with the HSF2-expressing cells (lanes 4 versus 5 and 7 versus 8). The transient expression of HSF2 or HSF1 was confirmed by Western blotting, indicating increased amounts of each protein in the respective transfected cells (Fig. 4B, lower panels). These results also suggest that HSF1, rather than HSF2, is activated to stimulate transcription of the hsp70 gene in hemin-treated K562 cells.

Effects of Constitutively Overexpressed HSF2—As we failed to detect hemin responsiveness of the K562 cells that had been transiently transfected with expression plasmids for GAL4–HSF2 fusion protein or intact HSF2 (Figs. 3 and 4), stable HSF2 overexpression cell lines were established to exclude the possibility that the expression levels were insufficient in those transient expression systems. The HSF2 expression levels in the stable lines used were 53-fold (clone 86–1) and 37-fold (clone 86–5) higher than that of the wild-type K562 cells (Fig. 5B). The overexpressed HSF2 resulted in constitutive HSE binding (Fig. 5C, lanes 1–3). However, the amounts of hsp70 and grp78 transcripts were not significantly elevated in these cell lines (Fig. 5A, lanes 1–3). Moreover, although the amount of HSF DNA binding increased further by hemin treatment (Fig. 5C, lanes 4–12), the extent of stimulation of hsp70 or grp78 transcription was not significantly higher than with the parent cells (WT) (Fig. 5A, lanes 4–12). On the contrary, overexpression of HSF2 seemed to be rather inhibitory to hemin-induced activation of hsp70 transcription (lanes 10–12). Thus, not only did the massive production of HSF2 fail to contribute to the hemin-induced activation of hsp70 transcription, but it was demonstrated that it can inhibit transcription perhaps by competing with HSF1 in binding to HSE on the hsp70 promoter.

DISCUSSION

We have shown that HSF1 is primarily involved in transcriptional activation of the hsp70 gene in hemin-treated K562 cells. It has been thought that HSF2 is probably the transcription factor responsible for hsp70 expression during hemin-induced differentiation because DNA binding of HSF2 increases upon hemin treatment. However, transiently overexpressed GAL4-BD–HSF2 chimeric protein or intact HSF2 failed to respond to hemin and activate transcription of the reporter gene containing a GAL4 binding site or HSE, respectively (Fig. 4A). Furthermore, in stably HSF2-overexpressing cells, the control or hemin-induced levels of hsp70 transcription was not significantly higher than in the mock-transfected cells (Fig. 5), despite the constitutive HSF2 binding to HSE (Figs. 2 and 5). On the other hand, GAL4-BD–HSF1 chimeric protein responded to both heat shock and hemin treatment and stimulated expression of the reporter gene (Fig. 3). Transiently overexpressed HSF1 also augmented the hsp70 transcription of the HSE-containing luciferase reporter in a dose-dependent manner (Fig. 4A). In addition, the majority of hemin-induced HSE binding complexes was shown to be HSF1 (Fig. 2), which presumably was partially phosphorylated as observed in heat-shocked cells (Fig. 1B). These results taken together strongly suggest that HSF1 but not HSF2 primarily mediates the hemin-induced transcriptional activation of the hsp70 gene in K562 cells.

The results presented here are apparently inconsistent with the previous reports. Sistonen et al. (21) reported that the hemin-induced HSE binding complexes observed in gel mobility shift analysis completely disappear with HSF2 antisera but are only slightly affected with HSF1 antisera. They also found that overexpression of HSF2-α isoform, which had been shown to function as a transcription activator (37), enhanced hemin-induced heat shock gene expression (33). It should be noted that HSF2 we used here was HSF2-α isoform but not β isoform, which had been reported to suppress hemin-induced expression of HSP70 (33).

These apparent contradictions might be explained on the basis of heterogeneity of K562 cells. Among the three known subtypes of K562 cells (38), we used B-subtype, which has a moderate responsiveness to hemin to differentiate. The HSF responsiveness to hemin probably varies among K562 cells maintained in different laboratories. As Sistonen et al. (32) also observed slight but significant trimer formation and partial phosphorylation of HSF1 upon hemin treatment, it is likely that their cell line was barely able to activate HSF1 in response to hemin. The slightly activated HSF1 might be enough to stimulate hsp70 transcription. In any event, the findings that hsp70 transcription does not increase in HSF2-overexpressing K562 cells despite its constitutive DNA binding (Fig. 5 and Ref.

**Fig. 5.** Effects of stably overproduced HSF2 on response to hemin. Parental cells (WT) and two stable HSF2-overproducing cell lines (86–1 and 86–5) were treated with 0, 15, 30, or 60 μM hemin for 12 h (lanes 1–12) or heat shocked for 30 min at 42 °C (lanes 13–15). A, 10 μg of total RNA was subjected to Northern blot analysis using hsp70, grp78, and glyceraldehyde-3-phosphate dehydrogenase probes. B, 20 μg of whole cell extracts were separated on 8% SDS-polyacrylamide gel electrophoresis, and Western blot analysis was performed using anti- HSF2 (αHSF2α) serum. C, HSE binding activities were analyzed by gel mobility shift assay. CHBA, constitutive HSE binding activity.
Hemin-induced HSF1 Activation

33) suggest that the acquisition of HSF2 DNA binding activity is insufficient and that an additional regulatory step is required to activate transcription of heat shock genes as in the case of HSF1 (39–42). It is possible that the cell line we used lacks the activity to convert the DNA binding form of HSF2 to a transcriptionally competent state. Alternatively, HSF2 may not bind to HSF2 or the hsp70 promoter in our K562 cell line as seen in untransfected F9 cells (23). In any case, HSF2 activation may not be essential during differentiation of K562 cells because apparent hemoglobin synthesis was observed in our experimental conditions (data not shown).

Proteasome inhibitors including peptide aldehyde N-acetyl-leucyl-leucyl-norleucinal and lactacystin induce HSF1 trimerization and hsp70 transcription (45), suggesting that accumulation of short-lived proteins that are normally degraded by proteasome induces hsp70 transcription via activation of HSF1. Interestingly, hemin can also inhibit ATP-dependent ubiquitin-dependent proteolysis at 25 μM (43, 44), which is comparable with what we employed to induce differentiation of K562 cells. It seems likely, therefore, that hemin can activate HSF1 through inhibiting proteolysis, leading to hsp70 transcription. Furthermore, the observation that GRP78 was induced by hemin with a kinetics similar to that of HSP70 induction can be explained by the recent finding that endoplasmic reticulum-resident chaperones (e.g. GRP78), as well as HSP70, are induced by various proteasome inhibitors (46).

We thus conclude that the HSP70 induction in hemin-treated K562 cells is primarily mediated by HSF1, which is presumably activated upon accumulation of abnormal proteins resulting from inhibition of proteasome activity. Although we failed to demonstrate any positive contribution of HSF2 to hemin-induced hsp70 transcription in K562 cells, it remains quite possible that HSF2 plays some roles during early development and differentiation. Because the inability of HSF2 to activate transcription from promoters carrying HSE has also been reported with other systems, including embryonal carcinoma cells (23) and early embryos (24), some unknown steps or conditions must be required for HSF2 transactivation capability. We recently suggested that HSF2 is negatively regulated in K562 cells by unknown factor(s) interacting with the negative regulatory motif adjacent to the C-terminal transactivation domain of HSF2 (25). The gel mobility shift analyses indicated that the presumptive factor(s) are able to interact with a DNA binding form of HSF2 (25). Further characterization of this and other factor(s) would provide a promising approach in elucidating the mechanism of HSF2 activation.

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REFERENCES

1. Lindquist, S., and Craig, E. A. (1988) Annu. Rev. Genet. 22, 631–677
2. Bensaude, O., and Morange, M. (1983) EMBO J. 2, 173–177
3. Bensaude, O., Bahin, C., Morange, M., and Jacob, F. (1983) Nature 305, 331–333
4. Zakeri, Z. F., and Wolgemuth, D. J. (1987) Mol. Cell. Biol. 7, 1791–1796
5. Milarski, K. L., and Morimoto, R. I. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9517–9521
6. Rabindran, S. K., Giorgi, G., Clos, J., and Wu, C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6906–6910
7. Schuetz, T. J., Gallo, G. J., Sheldon, L., Tempst, P., and Kingston, R. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6911–6915
8. Sarge, K. D., Zimarino, V., Holm, K., Wu, C., and Morimoto, R. I. (1991) Genes Dev. 5, 1962–1971
9. Nakai, A., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 1983–1997
10. Nakai, A., Tanabe, M., Kawazoe, Y., Inazawa, J., Morimoto, R. I., and Nagata, K. (1997) Mol. Cell. Biol. 17, 469–481
11. Sarge, K. D., Murphy, S. P., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 1392–1407
12. Baler, R., Dahl, G., and Voellmy, R. (1993) Mol. Cell. Biol. 13, 2486–2496
13. Nakai, A., Kawazoe, Y., Tanabe, M., Nagata, K., and Morimoto, R. I. (1995) Mol. Cell. Biol. 15, 5268–5278
14. Tanabe, M., Nakai, A., Kawazoe, Y., and Nagata, K. (1997) J. Biol. Chem. 272, 15389–15395
15. Loizzo, C. B., and Loizzo, B. B. (1975) Blood 45, 321–334
16. Loizzo, B. B., Loizzo, C. B., Bamberger, E. G., and Felui, A. S. (1983) Proc. Soc. Exp. Biol. Med. 166, 546–550
17. Andersson, L. C., Jokinen, M., and Gahmberg, C. G. (1979) Nature 278, 364–365
18. Rassow, T., Clegg, J. B., Higgs, D. R., Jones, R. W., Thompson, J., and Weaberthal, D. J. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 348–352
19. Singh, M. K., and Yu, J. (1984) Nature 309, 631–633
20. Theodorakis, N. G., Zand, D. J., Kotelkova, P. T., Williams, G. T., and Morimoto, R. I. (1989) Mol. Cell. Biol. 9, 3166–3173
21. Sistonen, L., Sarge, K. D., Phillips, B., Abravaya, K., and Morimoto, R. I. (1992) Mol. Cell. Biol. 12, 4104–4111
22. Murphy, S. P., Gotzowski, J. J., Sarge, K. D., and Phillips, B. (1994) Mol. Cell. Biol. 14, 5309–5317
23. Morgan, W. D., Williams, G. T., Morimoto, R. I., Greene, J., Kingston, R. E., and Tjian, R. (1987) Mol. Cell. Biol. 7, 1129–1138
24. Sistonen, L., Sarge, K. D., and Morimoto, R. I. (1994) Mol. Cell. Biol. 14, 2087–2099
25. Leppa, S., Pirkkala, L., Saarento, H., Sarge, K. D., and Sistonen, L. (1997) J. Biol. Chem. 272, 15283–15288
26. Catto, J. J., Kline, M., and Morimoto, R. I. (1996) J. Biol. Chem. 271, 3355–3358
27. Xia, W., and Voellmy, R. (1997) J. Biol. Chem. 272, 4094–4102
28. Green, M., Schuetz, T. J., Sullivan, E. K., and Kingston, R. E. (1995) Mol. Cell. Biol. 15, 3354–3362
29. Goodson, M. L., Park-Sarge, O.-K., and Sarge, K. D. (1995) Mol. Cell. Biol. 15, 5288–5293
30. Dimery, I. W., Ross, D. D., Testa, J. R., Gupta, S. K., Felsted, R. L., Pollak, A., and Bachur, N. R. (1983) Exp. Hematol. 11, 601–610
31. Hensold, J. O., Hunt, C. R., Calderwood, S. K., Housman, D. E., and Kingston, R. E. (1990) Mol. Cell. Biol. 16, 1600–1608
32. Price, B. D., and Calderwood, S. K. (1991) Mol. Cell. Biol. 11, 3365–3368
33. Jurvich, D. A., Sistonen, L., Kroes, R. A., and Morimoto, R. I. (1992) Science 255, 1240–1245
34. Bruce, J. L., Price, B. D., Coleman, C. N., and Calderwood, S. K. (1993) Cancer Res. 53, 12–15
35. Etlinger, J. D., and Goldberg, A. L. (1980) J. Biol. Chem. 255, 4563–4568
36. Haas, A. L., and Rose, J. A. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6845–6848
37. Zhou, M., Wu, X., and Ginsberg, H. N. (1996) J. Biol. Chem. 271, 24769–24775
38. Bush, K. T., Goldberg, A. L., and Nigham, S. K. (1997) J. Biol. Chem. 272, 9086–9092