Salicylate Triggers Heat Shock Factor Differently than Heat*

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Sodium salicylate has the unusual property of partially inducing the human heat shock response (Jurivich, D. A., Sistonen, L., Kroes, R., and Morimoto, R. I. (1992) Science 255, 1243-1245). Salicylate induces the DNA binding state of the human heat shock transcription factor (HSF), but this is insufficient to elevate heat shock gene expression. Because it is not known how HSF enhances heat shock gene expression, further analysis of the transcriptionally inert, salicylate-induced HSF was undertaken to potentially identify components of the heat shock response that are necessary for full transcriptional induction. Like thermal stress, exposure of HeLa cells to salicylate led to the induction of HSF1 into a DNA-bound state. However, continued exposure of cells to salicylate, HSF1 DNA binding attenuated much more rapidly than a continuous heat shock. Western blot analysis revealed that the salicylate-induced form of HSF1 was not hyperphosphorylated like the heat-induced form. Furthermore, supershifts of the HSF1 bound to an heat shock element (HSE) oligonucleotide by monoclonal antibodies to phosphoamino acids revealed that salicylate induced threonine phosphorylation of HSF1, whereas heat led to a predominance of HSF1 serine phosphorylation. These data suggest that salicylate-independent signals are necessary to convert HSF1 into a transactivator of heat shock gene expression and that brief acquisition of DNA binding by this factor is insufficient to maximally enhance transcription.

Pharmacological agents can alter gene expression. This property can be useful for understanding normal controls of transcription as well as providing insight into how drugs exert their therapeutic effect at the molecular level. Recently, anti-inflammatory drugs such as sodium salicylate have been found to alter specific transcriptional controls in human cells, as well as in plants and bacteria (1-4). How salicylate exerts its effect on the transcriptional machinery is not fully understood. Several studies indicate that drug-induced perturbations of DNA-binding proteins that act as enhancers or silencers of gene expression may be one anti-inflammatory drug target (1-2). Because the primary action of salicylate and other non-steroidal anti-inflammatory compounds (NSAIDs) has been attributed to disruption of eicosanoid acid metabolism (5), these agents could modify gene expression indirectly by modulating the levels of prostaglandins, leukotrienes, and other associated lipids. Alternatively, salicylate and NSAIDs may regulate gene expression by altering certain components of cellular signal transduction such as G-regulatory proteins (6). Finally, as these drugs have the capacity to bind proteins, an additional consideration is that DNA-binding proteins are directly affected by one or more NSAIDs.

Compelling evidence for salicylate in regulating gene expression has been identified in plant cells responding to viral injury (7-9). Induction of pathogen resistance genes is mediated by accumulation of salicylate both locally and systemically in disease-ridden plants. Whether salicylate directly induces transactivators of gene expression is not certain. A recent study has implicated salicylate's effect on gene expression through an indirect mechanism. By binding and inhibiting catalase, salicylate causes hydrogen peroxide to increase, which in turn induces expression of defense-related genes (10). Salicylate has also been found to stimulate gene expression in bacteria associated with antibiotic resistance (4).

In contrast to stimulating transactivation of some genes, salicylate can also inhibit gene expression. In plants, certain genes expressed during leaf wounding are inhibited by salicylate (11). Interferon-induced HLA-DR expression is altered by salicylate in human cells (12). Both these observations suggest that salicylate has the capacity to selectively block gene expression in addition to activating molecular switches for enhanced gene expression.

Given the relationship of salicylate to induction of genes in response to cellular injury in plants, recent investigations have been directed toward the possibility that this agent mimics signals in human cells associated with physiological stress. Thus far, salicylate has been found to affect two stress-associated transcription factors, NFκB and the heat shock transcription factor, HSF (1, 2). NFκB is induced by inflammation, viral, and UV-induced stress (13, 14). Salicylate apparently blocks NFκB by sustaining IκB levels and thus preventing translocation of the transcription factor to the nucleus (2). In contrast to its effects on NFκB, salicylate was found to induce HSF into a DNA binding state without enhancement of heat shock gene expression (1). The partial activation of the heat shock response by salicylate suggests that signals in addition to HSF multimerization are necessary to fully transactivate heat shock gene expression. This factor is underscored by the ability of heat shock to convert the salicylate-induced HSF into a transcriptionally competent enhancer of heat shock gene expression.

Several possibilities may account for salicylate's inability to fully induce the heat shock response. Because there are several known members of the heat shock factor gene family, it is possible that salicylate does not activate the heat-inducible factor, HSF1. Alternatively, salicylate-induced HSF1 may not be post-translationally modified in the same manner as the heat-inducible factor. Furthermore, salicylate may induce the

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1 The abbreviations used are: NSAID, non-steroidal anti-inflammatory compound; HSF, heat shock transcription factor; EMSA, electromobility shift assay(s); EMSSA, electromobility supershift assay(s); HSE, heat shock element; PAGE, polyacrylamide gel electrophoresis.
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DNA binding domain of HSF, but it may not affect the transactivation domain. In examining these possibilities, we found both salicylate and heat induced HSF1- and inducible HSF2/DNA binding. Despite induction of the same transcription factor as heat, the salicylate-induced form of HSF1 exhibited different properties. The duration of binding activity in vivo is different and the salicylate-induced form of HSF1 does not appear to undergo extensive phosphorylation as does the heat-inducible form. Phosphothreonine modification of HSF1 by salicylate is noted, whereas heat shock primarily causes phosphoserine modification of HSF1. Furthermore, the salicylate-induced HSF1 appears to be extensively associated with heat shock protein 70 (hsp70), a factor that may explain the incomplete activation of the human heat shock response by this antiinflammatory compound. These data suggest that both the duration of HSF1-DNA binding and additional modifications of HSF1 are linked to enhancement of heat shock gene expression.

MATERIALS AND METHODS

Cell Culture and Whole Cell Extracts—HeLa S3 cells were cultured at 37 °C in a spinner flask containing jokilik’s minimum essential medium and 5% calf serum with gentamycin sulfate and amphotericin B. Heat-shocked cells at various time points were obtained by submerging a capped and parafilm sealed 15-ml conical centrifuge tube into a 42 °C prewarmed circulating water bath. Cells were treated at 37 °C with various concentrations of sodium salicylate by dilution of a filter-sterilized 1 mM stock solution in phosphate-buffered saline without magnesium or calcium (PBS). Cells were adjusted to 4 x 10^6/ml in jokilik’s minimum essential medium containing 20 mM Hepes, pH 7.0, prior to heat shock or salicylate treatment. Whole cell extracts were obtained by washing cells in cold PBS and freeze fracturing the cell pellet in buffer containing 400 mM NaCl, 10% glycerol, 10 mM Tris, pH 8.0, 1 mM EDTA, and a mixture of the protease inhibitors phenylmethylsulfonyl fluoride, dithiothreitol, leupeptin, and pepstatin (Sigma). Protein concentrations of whole cell extracts were determined for 30 min at 32°C by Bradford assay (Bio-Rad).

Electromobility Shift Assays (EMSA)—EMSA were performed as described previously (15) utilizing a 32P-radiolabeled oligonucleotide that corresponds to the HSE of the human hsp70 gene. Electromobility supershift assays (EMSSA) were performed in a similar fashion to EMSA, although a lower percentage of polyacrylamide was employed (3.0%) in some assays and the HSF-HSE complex was subjected to longer volt-hours (800 V-h). Whole cell extracts (10 μg of protein) were incubated with antibodies up to 30 min at 21 °C prior to the addition of the reaction mixture containing the radiolabeled HSE. Various concentrations of antibodies were tested in each assay, and unless otherwise specified, 1.2 dilutions of the antibody relative to whole cell extracts are reported here. Antibodies employed in EMSA included polyclonal antiserum to recombinant heat shock factor 1 and 2 (16, 17), and monoclonal antibodies to phosphoserine, phosphothreonine, phospho-tyrosine (clones PSR-45, PTR-8, and PT-66, Sigma), hsp70 (C92F3A-5, StressGen; and RPN1197, Amersham), and hsp90 (16F1, StressGen).

Stable antibody solutions were 1 mg/ml for hsp70, hsp90, and goat anti-mouse IgG antibodies (Southern Biotechnology, 1010-01), whereas phosphoamino acid antibody stock solutions were 7.3 mg/ml. A 1 mg/ml stock solution of cycloheximide (Sigma) in ethanol was made and used at a final concentration of 10 μg/ml for cell preincubation.

Gene Expression—Nuclear run-on analysis was performed as described previously and transcription rates were determined by scanning densitometry and expressed numerically as a percentage of HSF1-DNA binding contained in 10 μg of protein from whole cell extracts of heat-shocked cells.

Immunoprecipitation of 32P-labeled HSF1 from HeLa S3 cells was performed after 1 x 10^6 cells were exposed to 750 μCi of inorganic, radiolabeled phosphorus in phosphate-free Dulbecco’s modified Eagle’s medium for 6 h. Cells were washed three times in PBS before treatment with 20 mM salicylate or a 42 °C heat shock for 30 min. After treatment, cells were washed once in PBS and the pellet was solubilized in 100 μl of boiling 1 X SDS solubilization buffer (10% glycerol, 2% SDS, 5% mercaptoethanol, 50 mM Tris, pH 6.8). Extracts were sonicated for 10 s and diluted 1:10 with NTE buffer (10 μg NaCl, 10 μg Tris-HCl, 1 mM EDTA). After a 30-min incubation of the lysate with 50 μl of a 50% solution of Sepharose-protein A in NTE buffer, the clarified extract was incubated 2 h with 2 μl of polyclonal rabbit serum specific for HSF1. A total of 2 μl of goat anti-rabbit IgG (1 mg/ml, Southern Biotechnology, 4010-01) was added to each extract for 30 min, followed by 70 μl of Sepharose-protein A solution for 60 min. The Sepharose-protein A was pelleted by centrifugation and washed six times with NTE buffer. The immunoglobulin-HSF1 complexes were solubilized in boiling 2 X SDS buffer (35 μl) and then boiled in distilled H2O (35 μl) and the fractions combined for a total volume of 70 μl.

RESULTS

HeLa S3 cells were exposed to different concentrations of salicylate, and whole cell extracts were made to analyze HSF-DNA binding activity by EMSA. In Fig. 1A, HSF-DNA binding activity in the salicylate-treated cells was measured by scanning densitometry and expressed as a percentage of HSF-DNA binding induced by a 42 °C heat shock. 20 mM salicylate induced HSF-DNA binding comparable to a 42 °C heat shock, while lower concentrations produced less HSF-DNA binding in a dose-dependent fashion.

To test whether any HSF-DNA binding activity induced by salicylate led to increased heat shock gene expression, nuclei were harvested at numerous time points for run-on analysis. Fig. 1B shows that a 42 °C heat shock induces hsp70 gene expression within 35-30 min, whereas none of the salicylate treated cells exhibited increased transcription of this gene. Because salicylate-induced HSF-DNA binding did not enhance RNA polymerase II activity for heat shock genes, additional studies were undertaken to identify how heat-induced HSF-DNA binding was different from that in salicylate-treated cells.

To examine whether salicylate was inducing the heat-regulated isoform of HSF, antibodies specific for HSF1 and HSF2 were preincubated with whole cell extracts prior to gel shift binding assay. Fig. 2A shows that HSF1 antibodies supershifted both the heat- and salicylate-induced HSF-HSE binding, whereas HSF2 antibodies had no effect. Thus, salicylate induces the same isoform of HSF as heat shock.

Because previous studies showed cycloheximide-dependent and -independent pathways of HSF activation, HeLa S3 cells were incubated with cycloheximide (10 μg/ml) prior to stimulation with salicylate. Fig. 2B shows that cycloheximide inhibits HSF-DNA binding activity when cells are exposed to 20 mM salicylate. Previous studies demonstrated the initial induction of HSF-DNA binding by heat was not affected by cycloheximide (17), thus indicating that the salicylate-inducible form of HSF1 responds differently than the heat-inducible form when protein synthesis is inhibited.

Additional differences were noted in the salicylate- and heat-inducible forms of HSF1 when HSF-DNA binding activity was examined over time. As can be seen in Fig. 3, both temporal activation and maintenance of HSF by salicylate at 37 °C differed from a 42 °C response. HSF-DNA binding activity
reached a maximum within 30-40 min during heat shock as compared with 20-30 min when cells were treated with salicylate. Despite continuous exposure to heat, HSF-DNA binding activity attenuated over a 3-4 h period, as has been previously reported (19). In contrast to the heat shock response and despite the continued presence of 20 mM salicylate, HSF-DNA binding activity attenuated rapidly. Within 90 min there was little to no HSF-DNA binding activity in the salicylate-treated cells. Even though salicylate-induced HSF-DNA binding activity attenuated rapidly, the in vitro dissociation of HSF-DNA binding of salicylate-treated cells was not different from that in heat-shocked cells (data not shown).

Additional in vitro analysis of salicylate-induced HSF1 was undertaken to identify potential differences relative to the heat-inducible form. When analyzed by ion-exchange chromatography, salicylate-induced HSF multimeric complexes or HSF-protein complexes eluted differently than the heat-induced factor. Fig. 4A shows that salicylate-induced HSF begins to elute from a DEAE column at a lower salt concentration than...
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A 42 °C heat shock for 30 min increased $^{32}$PO$_4$ incorporation by nearly 20-fold, whereas salicylate treatment increased radiolabel incorporation 2.6-fold.

To further examine possible differences between heat-shocked and salicylate-treated cells, supershifted HSF1-DNA complexes were sought in whole cell extracts treated with monoclonal antibodies to phosphoamino acids serine, threonine, and tyrosine. As seen in Fig. 5, antibodies to phosphoserine interfere with heat-inducible HSF1-DNA binding and cause a diffuse supershift of the HSF-HSE oligonucleotide complex within a 10-min heat shock at 42 °C. In extracts from salicylate-treated cells the phosphoserine antibodies had no effect on HSF1-DNA binding activity or mobility. However, phosphothreonine antibodies caused a marked supershift of the salicylate-induced HSF1. These antibodies appeared to slightly diffuse the otherwise distinct HSF-HSE banding observed in extracts from heat-shocked cells, but there was no frank retardation of the HSF-HSE complex in the gel as was observed with extracts from salicylate-treated cells. Phosphotyrosine antibodies had little to no effect on migration of either the salicylate- or heat-induced HSF1.

In addition to altered patterns of post-translational modifications of HSF1, some observable differences between the heat- and salicylate-inducible forms of HSF1 might be attributable to protein interactions with HSF. Indirect assessment of this possibility was approached by subjecting HSF-HSE oligonucleotide complexes to electrophoresis in lower percentage polyacrylamide gels for longer periods of time than usually employed for EMSA. Utilizing this approach, salicylate-induced HSF-HSE complexes migrated more slowly than heat-induced complexes (Fig. 6), suggesting that despite acquisition of DNA binding, the salicylate-induced HSF was associated with another protein that impeded its migration in the gel. One known candidate interacting with HSF1 is hsp70 (20). To examine the relationship of hsp70 binding to HSF1, an antibody specific for the inducible form of hsp70 was incubated in whole cell extracts prior to EMSA. Fig. 6 shows that antibody to hsp70 "supershifts" nearly all of the salicylate-induced HSF1-DNA binding complexes, whereas it only partially supershifts some of the heat shock-induced complexes. Antibodies to hsp90 did not appear to alter the HSF-HSE migration when mixed with either the salicylate- or heat shock-induced HSF. Antibody to $\gamma$-immunoglobulin, which was used as a control for nonspecific antibody-HSF interaction, had no effect in supershifting the HSF1-DNA complex either.

DISCUSSION

Salicylate is one of the few known inducers of HSF-DNA binding activity that does not enhance transcription of heat shock genes at 37 °C. None of the salicylate concentrations that increased HSF-DNA binding activity induced hsp70 gene expression. This dissociation of HSF-DNA binding from gene expression is quite different from heat shock, where hsp 70 gene expression can be induced to various levels depending upon the degree and duration of thermal stress (19). In HeLa S3 cells, a 42 °C heat shock leads to maximal heat shock gene expression by 30–40 min. HSF-DNA binding activity reaches an apex at
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Fig. 5. Electromobility supershift assay of HSF-HSE binding activity in the presence of monoclonal antibodies to phosphoserine, phosphothreonine, and phosphotyrosine. Whole cell extracts were made from HeLa S3 cells that were heat-shocked (HS) 10 min or salicylate-treated (SA) 30 min, and the extracts were incubated with the monoclonal antibodies prior to initiation of HSF1 binding with a radiolabeled HSE oligonucleotide. HSF represents the HSF-HSE complex, and NS is the nonspecific HSE-protein band. Supershifted HSF-HSE complexes are noted closer to the top of the autoradiograph than HSF-HSE complexes formed in the absence of antibodies. Incubation of the heat shock whole cell extract with anti-phosphoserine antibody led to both a “knock-out” or loss of HSF-HSE binding and a supershifted complex.

Fig. 6. EMSSA of HSF-HSE binding activity employing monoclonal antibodies to hsp70 and hsp90. Whole cell extracts of HeLa S3 cells were obtained after a 120 min heat shock at 42 °C or a 40 min treatment at 37 °C with 20 mM sodium salicylate. Extracts from these cells were preincubated with antibody to hsp70 (C92, StressGen) and hsp90 (16F1, StressGen). These extracts were compared to reaction mixtures that either had no antibody, but were diluted by an equivalent volume with a 10 mM, pH 7 Tris buffer or were exposed to an antibody with no known reactivity to heat shock proteins or HSF1. In the latter case, either serum or polyclonal antibodies to γ-immunoglobulin (IgG) were tested and none of these affected the HSF-HSE complex. The lack of HSF-HSE reaction with antibody to immunoglobulin G (Southern Biotechnology) is shown in this representative experiment. The HSF-HSE complex is designated by HSF, the nonspecific HSE-protein complex is indicated by NS, and HSF-HSE complexes supershifted by hsp70 antibodies are noted by an arrow.

this time also. As HSF-DNA binding attenuates, the rate of heat shock gene expression also declines in a proportionate fashion. By comparison, salicylate can induce HSF-DNA binding up to 100% of that induced by a 42 °C heat shock and yet not result in the enhanced expression of heat shock genes. This observation suggests that salicylate can trigger HSF-DNA binding without inducing some other event that renders HSF transcriptionally competent.

Perhaps a simple explanation of salicylate’s inability to induce a transcriptionally competent factor is that it does not activate the same factor as heat shock. Several isoforms of heat shock factor exist (21, 22). Heat shock induces HSF1, whereas hemin induces HSF2 (23). Furthermore, transcription rates of the heat shock genes are substantially less when HSF2 is compared to the heat-inducible isoform, HSF1. Because salicylate-induced HSF reacts with antibodies directed against HSF1 and not HSF2, this observation indicates that salicylate affects the same transcription factor as heat. Salicylate either partially induces HSF1 modifications necessary for transactivation of the heat shock genes or it modifies HSF1 differently than heat shock.

Even though salicylate and heat induce the DNA binding state of HSF1, it is not clear that these stimuli share the same pathway of induction. Previous work has suggested that all inducers of heat shock genes share a common triggering mechanism, namely abnormal proteins (24). More recent work has indicated that HSF1 can assume a DNA-bound state simply in response to elevated temperatures (25), thus suggesting that temperature itself can regulate HSF1-DNA binding in addition to unspecified signals generated by abnormal proteins. Unlike heat, salicylate does not directly induce HSF1-DNA binding activity when examined by in vitro assays, thus implying an indirect action. Recently, salicylate has been found to inhibit catalase activity in plants, and it is thought that increased H2O2 levels stimulate trans-acting factors that enhance expression of the so-called defense related genes (10). A similar mechanism might be evoked in HeLa cells exposed to salicylate; however, we were unable to prevent salicylate-induced HSF1-DNA binding by preincubating cells with various antioxidants.

Another difference between heat and salicylate induction of HSF1 is revealed by the pretreatment of cells with cycloheximide. Blockage of protein synthesis prevents HSF1-DNA binding induced by 20 mM salicylate, whereas cycloheximide delays the heat induction of HSF1, but not overall binding levels (17). If one were to evoke the protein-damage model for triggering HSF1, then salicylate would trigger HSF1 through production of abnormal, newly synthesized polypeptides whereas heat would work through accumulation of damaged, preexisting proteins. The paradox here is that if the model of protein damage as a common mechanism of induction for heat shock gene expression is correct, then salicylate ought to fully induce the heat shock response. One interpretation is that damaged proteins can contribute toward the acquisition of HSF1-DNA binding, but additional signals are needed to trigger gene expression.

Additional signal(s) necessary for HSF1 to fully enhance heat shock gene expression are not fully understood. Even though HSF1 can bind to DNA, it may have to undergo additional modifications in order to be a transactivator of heat shock gene expression. These modifications may include i) release of a regulatory protein, ii) interaction with another DNA-binding protein, and iii) post-translational modifications.

In considering the role of a negative regulator of HSF1 function, some investigators have suggested hsp70 as a modulator of HSF1 function (26, 27). Hsp70 does not prevent HSF1 from binding DNA (28), but it could possibly block the transactivation domain of HSF1. Our observations with salicylate are consistent with the hypothesis that hsp70 bound to HSF1 suppresses transactivation of the heat shock genes. If one exam-

2 D. A. Jurivich, C. Pachetti, L. Qiu, and J. F. Welk, unpublished data.
vides HSF1-HSE complexes on low percentage polyacrylamide gel shift assays, the salicylate-induced HSF1 migrates differently than the heat-induced form and the former is almost completely supershifted by antibodies to hsp70. The ability of hsp70 antibodies to supershift most of the salicylate-induced HSF1 complexes (80% by scanning densitometry) suggests that salicylate is incapable of inducing a pool of HSF1 free of hsp70 such as seen during heat shock. An inference can be made from this finding that maximal expression of heat shock genes requires a nominal pool of HSF1, which is not associated with hsp70. According to this hypothesis, salicylate would have the capacity to trigger HSF1-DNA binding activity but would be unable to cause dissociation of hsp70 from the multimerized transcription factor. Unresolved is whether salicylate somehow increases the association of hsp70 with HSF1 or whether it does not promote the dissociation of preexisting hsp70 and HSF1 complexes. Thus far, we have not detected hsp70 in immunoblots of HSF1 from non-stimulated cells, but this observation does not preclude the possibility of transient interactions of hsp70 and HSF1 whereby salicylate shifts the equilibrium toward more stable interactions.

On the other hand, transcriptional incompetency of salicylate-induced HSF1 may be due to the lack of post-translational modifications or possibly inappropriate modifications of HSF1. Heat shock induces HSF1 phosphorylation (16, 29). This modification results in the altered mobility of HSF1 on SDS-PAGE putatively by phosphorylated residues displacing SDS binding which retard the mobility of this protein. When compared to the heat shock form of HSF1, the salicylate-induced form shows little to no retardation on SDS-PAGE. HSF1 incorporation of 32P subsequent to salicylate treatment is less than heat shock treatment. These data suggest that salicylate does not induce maximal HSF1 phosphorylation usually observed in heat shock samples. Whether increased phosphorylation of HSF1 is associated with maximal expression of heat shock genes is not certain. In yeast, a constitutively bound HSF1 becomes phosphorylated as heat shock gene expression increases. However, HSF phosphorylation has recently been associated with attenuation of HSF-DNA binding activity (30). Given that as little as one phosphorylated amino acid can confer transactivation of some transcription factors (31), it is possible that certain phosphorylated residues on HSF1 are associated with transactivation whereas others are linked to attenuation.

Interestingly, salicylate may actually induce an unique form of HSF1 phosphorylation as suggested by supershifted complexes of HSF1-HSE oligonucleotides in the presence of monodonal antibodies to phosphoamino acids. HSF1 induced by heat appears to interact primarily with phosphoserine and, to a lesser degree, phosphotyrosine antibodies. In contrast, salicylate-induced HSF1 leads to an HSF1-HSE complex that supershifts primarily with phosphothreonine and to a lesser degree with phosphotyrosine. The supershifting of the HSF-HSE complexes by monodonal antibodies to phosphoamino acids may be the result of HSF1 phosphorylation or some other phosphoprotein complexed with HSF1. In either case, it is curious that salicylate causes a dramatically different pattern of phosphorylation than heat shock. Our data suggest that salicylate may block a threonine-specific phosphatase, activate a threonine protein kinase, or a combination of both. On the other hand, salicylate may activate a serine/threonine kinase and the conformation of HSF1 may be such that only threonine residues are affected. Because salicylate-induced HSF1-DNA binding is so brief relative to heat shock, it is possible that threonine phosphorylation of HSF1 may regulate the duration of DNA binding activity.

One final consideration about the inability of salicylate to induce heat shock gene expression entails conformational changes in HSF1. As some investigators have reported HSF1 unfolding as a step toward multimerization (32), it is possible that further conformational changes in HSF1 are necessary to transactivate the heat shock genes. For example, the leucine zipper repeats located in the carboxy terminus of HSF1 have been cited as a domain of HSF1 possibly contributing to transactivation of heat shock genes (32). A sequential unfolding of different domains of HSF1 would first allow multimerization and then transactivation. The "hinge model" of HSF1 whereby the carboxyl terminus swings outward and unmasks the transactivation domain has been proposed in yeast where HSF1 is constitutively bound (30). In human cells, the unmasking of this regulatory domain could be insensitive to the action of salicylate. In this model, salicylate would induce HSF1 conformational changes but would be unable to create the proper signals for unmasking the transactivation domain. The inability of salicylate to induce this final step could account for a transcriptionally inert HSF1 that binds to the heat shock element.

While salicylate is a useful tool in dissecting the properties of HSF1 that are associated with maximal expression of the heat shock genes, we have also been interested in the effect of salicylate on the heat-inducible expression of the heat shock genes. We have found that concentrations of salicylate that by themselves do not induce HSF1-DNA binding activity will act synergistically with heat in regulating heat shock gene expression.2 These synergistic actions include enhancement of HSF1-DNA binding levels at physiologically relevant temperatures and concomitant increases in the rate of heat shock gene expression relative to thermal induction alone. These observations suggest that the action of salicylate is different under heat stress than at 37°C, and this may have important implications for the efficacy of salicylate and perhaps other NSAIDs in modulating inflammation and possibly increasing cellular protection through the heat shock response.

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