Communication

Two Functional Domains of Human Heat Shock Factor 1 Have Different Effects on Its DNA-binding Activity through Redox Changes

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Human heat shock transcription factor 1 (hHSF1) plays important roles in heat shock response. In unstressed human cells, HSF1 is present in an inactive monomer. After heat stimuli, hHSF1 forms homotrimer and gains the heat shock element (HSE)-binding activity.[1-4] Three function domains, including DNA-binding domain (DBD), trimerization domain (TD) and transcription activation domain (TAD), exist in hHSF1.[2]

Previous studies verified that redox changes could influence hHSF1 DNA-binding.[5,6] However, their roles of different domains remained unknown. Thus, we have built three kinds of hHSF1 proteins including: full-length hHSF1-α (amino acid 1-529), hHSF1-β (1-290) containing the DBD and TD, and hHSF1-γ (1-120) containing the DBD. Proteins were expressed in *E. coli* stains[7] and purified by His-tagged and Mono Q HP columns.[8] DNA-binding and trimerization activities were analyzed by electrophoretic mobility shift assay and electrophoresis gel in the presence of redox chemicals (diamide (DM), hydrogen peroxide (H_2O_2), dithiothreitol (DTT)).

In Figure 1, hHSF1-α was treated with redox chemicals and heat-activated. As a result, the addition of increasing amounts of DTT, a reducing agent, gradually increased the formation of heat-induced hHSF1-HSE complexes and homotrimer (lanes 2-4 in Figure 1A; lanes 2-3 in Figure 1B). However, when treated with oxidizing agents DM or H_2O_2, hHSF1-α showed an inhibitory effect on the process of DNA-binding and trimerization (lanes 5-10 in Figure 1A; lanes 4-7 in Figure 1B). These results strongly suggested that heat-induced HSE binding and trimerization activities of hHSF1 were regulated by redox potential.

To test the impact of the TAD on hHSF1-activation, we examined hHSF1-β under the same conditions as hHSF1-α. In Figure 2, hHSF1-β showed a strong HSE binding activity and formed a stable homotrimeric complex. Redox chemicals could not change its activities. These results suggested that heat stimuli could activate hHSF1-β; however, this process is not redox-dependent. Generally, redox regulation in proteins lies on the formation of disulfide bond.[9] Previous studies showed formation of intramolecular disulfide bond (between Cys153 and Cys373 [or Cys378]) inhibits heat-

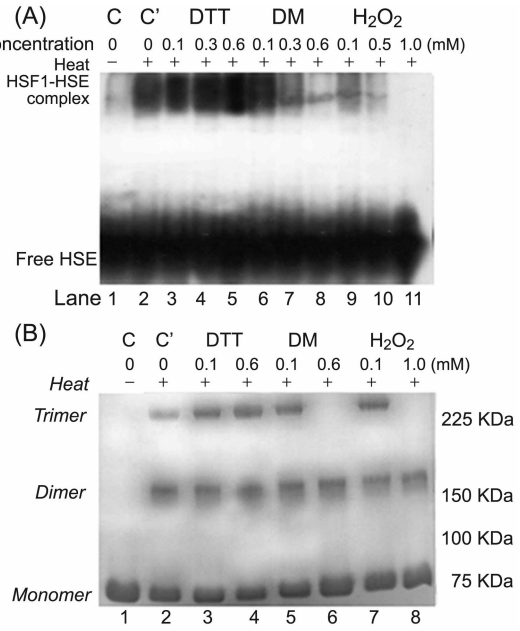

Figure 1. (A) The heat-induced DNA-binding activity of hHSF1-α (amino acid 1-529) in the presence of redox chemicals. *E. coli* strains were cell-cultured at 20 °C for 12 hours.[8] The indicated amounts of dithiothreitol (DTT), diamide (DM), and hydrogen peroxide (H_2O_2) were added to the hHSF1-α (4 °C, 10 min). After heat shock (42 °C, 1 hour), the protein was treated with heat shock element (HSE). The hHSF1-HSE complexes were analyzed by 5% native electrophoresis gel and visualized by autoradiography. (B) Trimerization activity of hHSF1-α in the presence of redox chemicals. The hHSF1 were treated with the same methods as Figure 1A. After heat-activation (42 °C, 1 hour), samples were mixed with 5 × loading buffer (without any redox chemicals) and boiled at 95 °C for 10 min to completely denature proteins.[5] Then, samples were loading into 6% (hHSF1-β in 12% and hHSF1-γ in 15%) electrophoresis gels. Following transferred to nitrocellulose membrane, proteins were analyzed by immunoblotting with anti-HSF1 antibody. C means not heat, C' means heat.

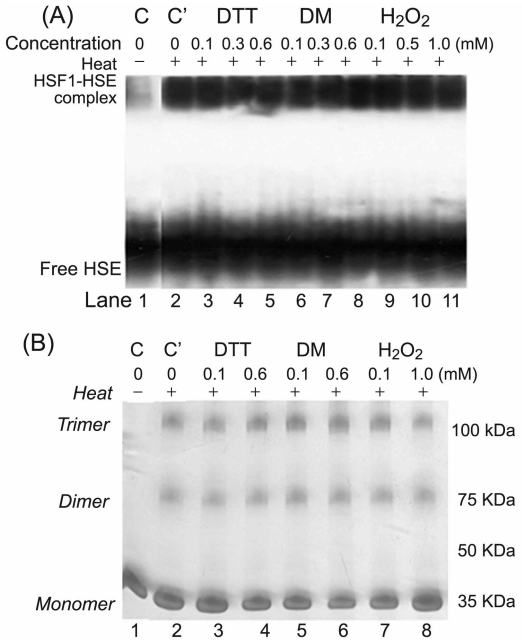

Figure 2. (A) and (B) are heat-induced DNA-binding and trimerization activities of hHSF1-β in the presence of redox chemicals. The experimental methods are the same as Figure 1(A) and (B).

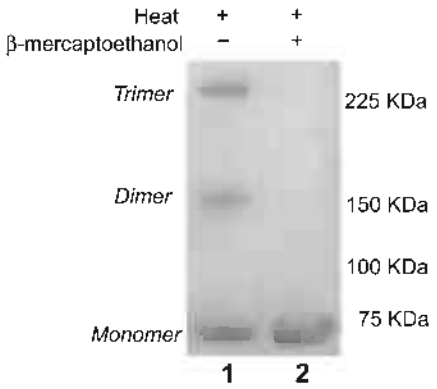

Figure 3. The hHSF1-α was heat-activated at 42 °C and then treated with 3 mM β-mercaptoethanol for 10 min (lane 2) or not (lane 1). Trimerization was analyzed by 6% SDS-PAGE.

induced DNA-binding activity.[10] Thus, lose of two cysteine residues 373 and 378 by deletion of TAD may result in the failure of redox regulation. Otherwise, hHSF1-α and -β could form trimer in SDS-PAGE without redox chemical (Figure 1B and 2B, lanes 2), suggesting that some strong interactions may be involved in hHSF1 trimerization. To verify whether redox-sensitive disulfide bond was involved in this strong interaction, we treated hHSF1-α with excess β-mercaptoethanol (3 mM), a reducing agent. In Figure 3, trimerization was completely reversed by β-mercaptoethanol, strongly suggesting the existence of disulfide bond in hHSF1-trimers.

To find the effect of the TD on hHSF1-activation, we repeated the same experiments with hHSF1-γ. In Figure 4, hHSF1-γ did not exhibit their DNA-binding and trimerization activities, suggesting that the DBD could not form a correct trimeric format even under stress conditions. The

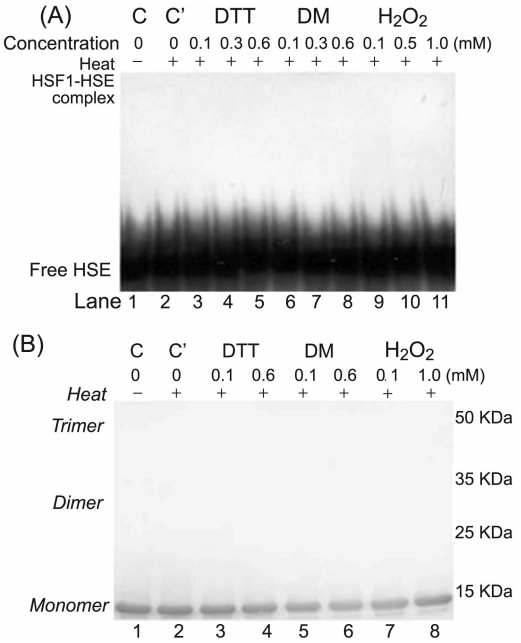

Figure 4. (A) and (B) are heat-induced DNA-binding and trimerization activities of hHSF1-γ in the presence of redox chemicals. The experimental methods are the same as Figure 1(A) and (B).

failure of hHSF1-γ trimerization suggested two possibilities: One is that TD was also essential in disulfide bond-mediated trimerization of hHSF1. Another is that C3 residue in TD is important for intermolecular disulfide bond formation.

DNA-binding activity of hHSF1 is redox-sensitive *in vitro*, suggesting that hHSF1 could play important roles in various physiological phenomena *in vivo*, since intracellular ROS (reactive oxygen species) level is very sensitive in response to different environmental stresses.[11] In this study, our experiment results showed that two domains (TD and TAD) of hHSF1 participate in redox-dependent DNA-binding activation. However, their roles in this process are different.

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References

1. Wu, C. *Annu. Rev. Cell Dev. Biol.* **1995**, *11*, 441.
2. Morimoto, R. I. *Genes Dev.* **1998**, *12*, 3788.
3. Voellmy, R. *Cell Stress Chaperone.* **2004**, *9*, 122.
4. Pirkkala, L.; Nykanen, P.; Sistonen, L. *FASEB J.* **2001**, *15*, 1118.
5. Manalo, D. J.; Liu, A. Y.-C. *J. Biol. Chem.* **2001**, *276*, 23554.
6. Ahn, S.-G.; Thiele, D. J. *Genes Dev.* **2003**, *17*, 516.
7. Ahn, S.-G.; Liu, P. C. C.; Klyachko, K.; Morimoto, R. I.; Thiele, D. J. *Genes Dev.* **2001**, *15*, 2134.
8. Soncin, F.; Prevelige, R.; Calderwood, S. K. *Protein Expres. Puri.* **1997**, *9*, 27.
9. Dietz, K. J.; Scheibe, R. *Physiologia Plantarum.* **2004**, *120*, 1.
10. Manalo, D. J.; Lin, Z.; Liu, A. Y.-C. *Biochemistry* **2002**, *41*, 2580.
11. Seo, H. R.; Chung, D. Y.; Lee, Y. J.; Lee, D. H.; Kim, J. I.; Bae, S. W.; Chung, H. Y.; Lee, S. J.; Jeoung, D.; Lee, Y. S. *J. Biol. Chem.* **2006**, *281*, 17220.