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Aromatic-Participant Interactions Are Essential for Disulfide-Bond-Based Trimerization in Human Heat Shock Transcription Factor 1†

Ming Lu,‡ Yun-Ju Lee,‡ Sung-Min Park,‡ Ho Sung Kang,§
Shin Won Kang,‡ Suhkmann Kim,‡ and Jang-Su Park*,‡

‡*Department of Chemistry and* §*Department of Molecular Biology, Pusan National University, 609-735 Busan, South of Korea*

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ABSTRACT: Heat shock transcription factor 1 (HSF1) is a central regulator in the heat shock response. However, its trimerization mechanism remains unclear. Here, we demonstrate that three conserved aromatic amino acids (Trp37, Tyr60, and Phe104) are essential for HSF1 trimerization. Point mutation and fluorescence spectroscopy experiments show that an intramolecular interaction between Tyr60 and α-helix 1 in the DNA-binding domain stabilizes the HSF1 structure upon heat stress. Furthermore, intermolecular aromatic−aromatic interaction between the Trp37 and Phe104 supports the approach with the Cys36 and Cys103. Thus, the existence of two differential interactions facilitates the formation of intermolecular disulfide bonds, leading to the heat-induced HSF1 trimerization.

All organisms have evolved essential systems to respond to different stresses such as heat shock. In metazoan, the heat shock response is mainly regulated by the heat shock transcription factor 1 (HSF1).[1] HSF1 is present in unstressed cells in an inactive monomeric form and becomes activated to an active trimer by heat or other stress stimuli (*1*−*4*). HSF1 trimerization and DNA-binding are considered critical processes in HSF1 activation. In response to heat stress, HSF1 readily forms a trimer in solution though interaction of its trimerization domain and is predicted to form a three-stranded coiled-coil (*5*, *6*). Activated HSF1 trimers constitutively bind DNA upstream of the heat shock promoter at sequences called the heat shock elements (HSEs), which are comprised of multiple inverted, nGAAn tandem repeats (*7*, *8*). We recently presented a novel, redox-dependent mechanism for the dual-regulation of human HSF1 (hHSF1) trimerization by two distinct sets of disulfide bonds (SS bond) with differing redox sensitivities (*9*). Importantly, the formation of three intermolecular SS bonds between the Cys36 and Cys103 residues located in the DNA-binding domain (DBD) is essential for HSF1 trimerization and DNA-binding (Figure 1A). The same SS bond was also found in mouse HSF1 (*10*). However, the mechanism by which Cys37 and Cys103 approach each other and form this intermolecular SS bond remains unknown, although our previous result (*9*) showed that intermolecular, hydrophobic, interaction-mediated, noncovalent trimerization is a precondition of SS-I bond formation. In this study, we found that hHSF1 homotrimerization is mediated by aromatic-participant interactions.

Previous X-ray crystallography and NMR spectroscopy studies revealed that highly conserved DBD of HSF, which contains three α-helices and four *β*-sheets, belongs to a member of the "winged" helix−turn−helix (wHTH) superfamily (*11*−*13*). A defining feature of this family is the central wHTH region composed of the second and third α-helices. Biochemical and genetic experiments suggested that the third helix is the DNA recognition site (*13*). Furthermore, the four *β*-sheets form a single antiparallel *β*-sheet, which closes off the hydrophobic core formed by the bundle. However, the roles of the first helix (H1) in HSF1-activation are still unknown. For this purpose, we compared the heat-induced trimerization and DNA-binding activities of wild-type hHSF1 with those of its H1 deletion (△H1) mutant (formed by deleting the amino acid sequence 13−29 of α-helix 1 in hHSF1) (Figure 1B,C). In this study, hHSF1 protein and all its mutants were expressed in *Escherichia coli* BL21 (DE3) strains and purified by our previous methods (*9*) (Figure S1, Supporting Information). Moreover, these purified proteins were examined by CD spectroscopy, and the result in Figure S2 (Supporting Information) shows that our mutants do not cause the conformational change of HSF1. Intermolecular SS bond-based trimer-formation was detected by nonreducing SDS−PAGE (Figure 1B), and the DNA-binding activity of hHSF1 was examined by the electrophoretic mobility shift assay (EMSA) (Figure 1C). Moreover, no reducing agent [dithiothreitol (DTT) or *β*-mercaptoethanol] was present in the 5× electrophoresis sample buffer for these SDS−PAGE experiments. Unlike wild-type hHSF1, the △H1 mutant that lacks H1 could not form a trimer and bind to

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*To whom correspondence should be addressed. Phone: +81-51-510-2294. Fax: +82-51-516-7421. E-mail: jaspark@pusan.ac.kr.

[1]HSF1, heat shock transcription factor 1; DBD, DNA-binding domain; HSE, heat shock element; SS bond, disulfide bond; FI, fluorescence intensity.

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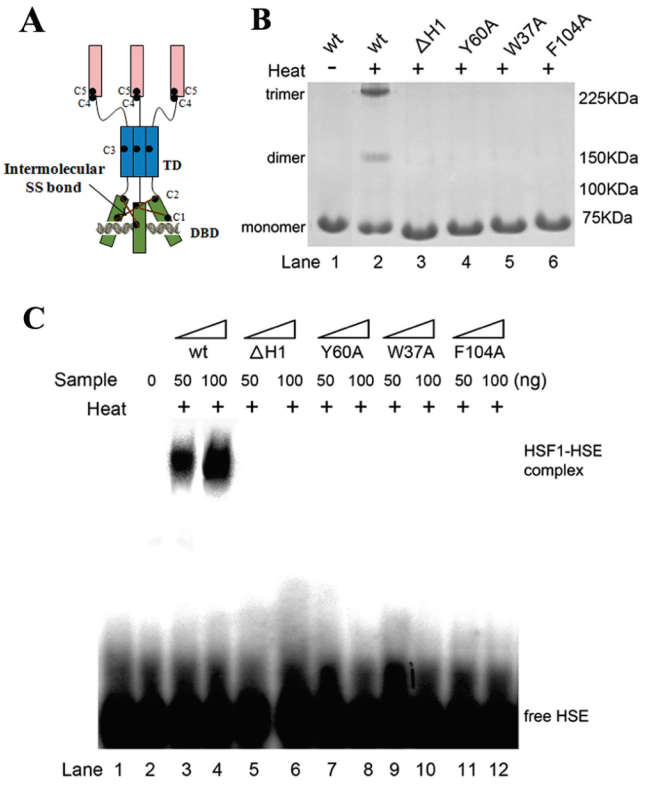

FIGURE 1: The heat-induced trimerization and DNA-binding activities of hHSF1 and its various mutants. (A) Diagrammatic illustration of an active hHSF1 trimer. DBD, the DNA-binding domain. TD, the trimerization domain. RD&TAD, the regulation and transcriptional activation domain. (B) Samples were heat activated (30 min at 42 °C), and their trimerization activities were analyzed by SDS−PAGE. (C) Samples were heat activated, and their DNA-binding activities were measured by EMSA using 32^{P}-labeled HSE (*9*). Lane 1 is the control.

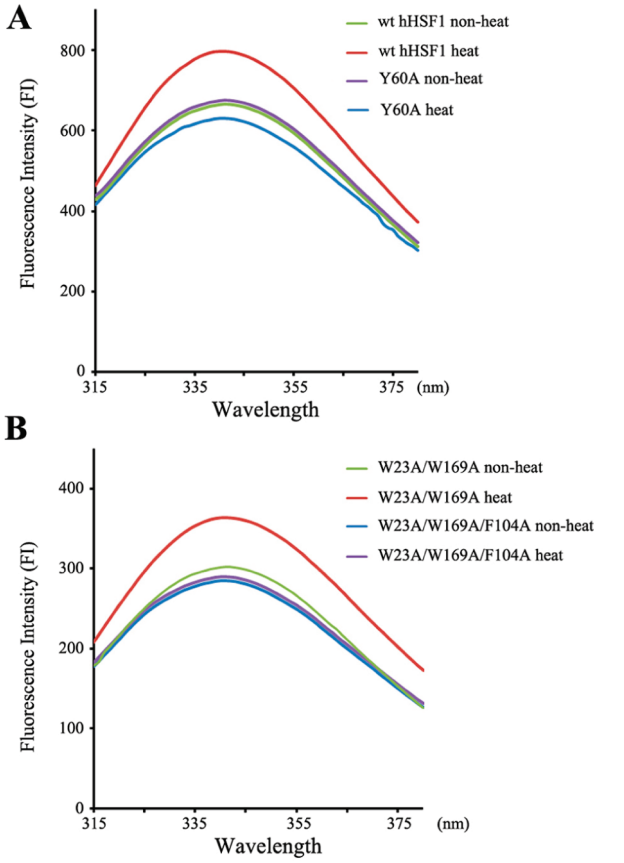

FIGURE 2: Trp-Fluorescence spectra of hHSF1 and its mutants. Fluorescence samples (200 μg/mL) were prepared in TGE buffer (50 mM Tris-HCl, 25% (v/v) glycerol, 1 mM EDTA, 1 mM DTT, pH = 7.5). For the selective excitation of Trp amino acid, a wavelength of 295 nm was used (the parameters were setup as $\lambda_{excitation}$ = 280 nm, $\lambda_{emission}$ = 315−380 nm).

HSE DNA (lanes 2−3 in Figure 1B and lanes 2−6 in Figure 1C). These results suggest that H1 is required for hHSF1 trimerization and DNA-binding, though it may not directly bind the HSE. Furthermore, the DBD structure obtained from *Drosophila* HSF (*14*) suggested that an aromatic side-chain of the highly conserved residue, Tyr92, located in the "turn" of the wHTH region is packed against the hydrophobic core, showing intramolecular interactions with the following helix1 (H1) residues: Ala48, Phe49, Lys52, and Leu56 (Figure S3, Supporting Information). These conserved residues are also found in hHSF1 (relative to residues Ala17, Phe18, Lys21, Leu25, and Tyr60) (*3*), suggesting that the same intramolecular interaction may exist in hHSF1. Thus, we generated an Y60A mutant to examine this interaction and its effect on hHSF1 activation. In the results, the Y60A mutant did not show heat-induced activities (lane 4 in Figure 1B and lanes 7−8 in Figure 1C), suggesting that the intramolecular interactions between the Y60 residues and H1 also existed in hHSF1. Furthermore, wild-type hHSF1 and Y60A mutants were examined by Trp-fluorescence spectroscopy that is used to identify the protein folding/unfolding (*15*). The wild-type hHSF1 after heat shock (42 °C, 5 min) had a higher fluorescence intensity (FI) than that in its native state (Figure 2A). This result suggested that hHSF1 is a thermophilic protein, because hHSF1 could be induced to enter into a more folded (or more compact) state, but not into an unfolded one. Unlike the wild-type hHSF1, heat stimuli did not increase the FI value of the Y60A mutant. Thus, these results demonstrated that the interaction between Y60A and H1 is essential for the heat-induced structural change of hHSF1 and that its breakdown could inhibit intermolecular SS-bond-based trimerization and DNA-binding.

The above-mentioned fluorescence spectra and trimerization assay results revealed that heat-induced hHSF1 trimerization increases the FI values. In addition, our previous study (*9*) proved that the trimerization of hHSF1 needs the connection of two cysteine residues (Cys36 and Cys103) from the different hHSF1 monomers. Coincidentally, two highly conserved aromatic amino acids (Trp37 and Phe104) are located close to these two cysteine residues (red dots in Figure S3, Supporting Information). According to NMR data from *Drosophila* HSF DBD (*14*), these residues (Cys36, Trp37, Cys103, and Phe104) are on the surface of whole hHSF1. To identify whether Trp37 and Phe104 are involved in intermolecular SS bond formation, W37A and F104A mutants were respectively performed by previous experiments. Both hHSF1 mutants lost their heat-induced trimerization and DNA-binding activities (lanes 5−6 in Figure 1B and lanes 9−12 in Figure 1C), suggesting that absence of two aromatic amino acids prevents the two cysteine residues from different hHSF1 monomers to approach one another. Burley and

Petsko (*16*) suggested that an aromatic pair will interact if the distance between the phenyl ring centroids is less than 7 Å. We therefore investigated whether an intermolecular, aromatic−aromatic interaction could induce the formation of an intermolecular disulfide bond. Phe104 is a hydrophobic amino acid. If a Trp37 residue approaches the Phe104 residue, the FI value of Trp37 will be increased. Three tryptophan residues (Trp23, Trp37, and Trp169) are found in human HSF1 (*3*). To examine the FI value of Trp37, we generated two hHSF1 mutants (one is W23A/W169A double mutant containing Trp37 and Phe104; another is W23A/W169A/F104A treble mutant containing Trp37 only). The W23A/W169A double mutant of hHSF1 showed higher fluorescence intensity under heat induced stress than it did in its nonheated state. In contrast, the W23A/W169A/F104A treble mutant did not exhibit such a change in its fluorescence intensity (Figure 2B). Thus, these results suggest that the disruption of the aromatic interaction between Trp37 and Phe104 inhibits the formation of the intermolecular SS bond and hHSF1 trimerization. Previous studies (*17*, *18*) demonstrated that intermolecular, noncovalent, hydrophobic (leucine zippers in the trimerization domain of HSF1) interactions enabled HSF1-trimerization. Our own previous findings (*9*) proved that this noncovalent trimerization is a precondition for intermolecular SS bond formation. Thus, heat stress first induces three hHSF1 monomers to interact with each other via their hydrophobic region in the trimerization domain, which suggests that the distance between the two aromatic amino acids is less than 7 Å. At the same time, the intramolecular interactions between H1 and Tyr60 stabilize the structure of hHSF1, which facilitates the interaction of the aromatic pair (Trp37 and Phe104). Finally, two cysteine residues from the different hHSF1 monomers connect with one another to form an intermolecular SS bond, which finally results in the formation of an active hHSF1 trimer (Figure 3).

Generally, most proteins gain (or lose) their functional activities by modulating some specific intramolecular interactions, such as hydrophobic interactions, hydrogen bonds, disulfide bonds, etc. However, in previous reports, there has been little evidence that intermolecular aromatic−aromatic interactions induce the formation of intermolecular disulfide bonds. Our findings showed that the interaction of three aromatic residues (Trp37, Tyr60, and Phe104) in hHSF1 is required to facilitate the formation of a trimer through intermolecular SS bonds, thus providing a unique model to understand protein activation (Figure 3). Furthermore, a recent study (*19*) found that although HSF1 plays remarkably potent modifier-like roles in the tumor-free survival of whole animals, it also regulates oncogenesis by either oncogene activation or tumor suppressor inactivation by modulating diverse cellular responses. Thus, a method of inhibiting the activation of HSF1 during carcinogenesis is essential for postponing the progression of cancer, because the lack of HSF1 is not lethal to body growth (*19*). Our findings provide a theoretical framework for inhibiting the lethal phenomenon of cancer.

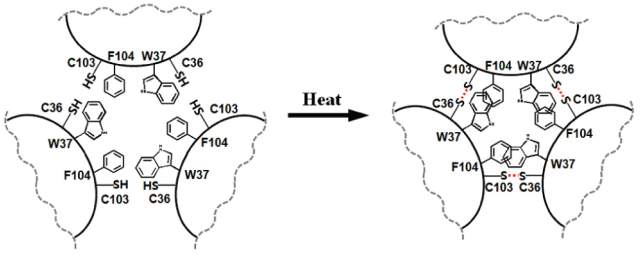

FIGURE 3: Diagrammatic illustration of intermolecular aromatic interactions between two phenyl-containing amino acids which induce the formation of intermolecular disulfide bonds.

SUPPORTING INFORMATION AVAILABLE

General experimental procedures, data showing the purified hHSF1 and its mutants (Figure S1), and figure showing the alignment of the amino acid sequences for the DBDs of human HSF1, *Drosophila* HSF, and *Kluyveromyces lactis* HSF (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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