http://dx.doi.org/10.5012/bkcs.2012.33.12.4201

Roles of His_{101} in DNA-Binding Domain of Human Heat Shock Factor 1 Under Acid pH Environment

Ming Lu, Bobae Kwon, Ziwei Chang, Hyeon-Gyeong Park, and Jang-Su Park*

Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan 609-735, Korea
**E-mail: jaspark@pusan.ac.kr*
Received July 6, 2012, Accepted September 6, 2012

Key Words : Human heat shock factor 1, Tryptophan-fluorescence spectroscopy, Acid pH, Histidine

The cellular response to acute and proteotoxic stresses, historically called "heat shock response" (HSR), is strongly conserved throughout all known organisms.[1,2] Heat shock factor 1 (HSF1) was first characterized as a major transcription factor during HSR, by its potentialities to regulate the expression of heat shock proteins (HSPs, also known as molecular chaperones).[1] In most tissues and cell types, HSF1 is constitutively expressed, and is kept inactive in the absence of stress. Under the abnormal conditions, HSF1 homo-trimer is formed by three monomeric ones, and exhibits its DNA-binding and post-translational activities (inducing the expression of downstream HSP genes).[2] Recent studies have reported that HSF1 plays a powerful multi-faceted role in tumor growth and response to cancer therapy.[3]

Cancer needs an acid and low oxygen environment to survive and flourish within.[4] Terminal cancer patients are over 1000 times more acidic than normal healthy people. Because in the absence of oxygen, cellular glucose undergoes fermentation to lactic acid, which causes the pH of the cell (intracellular pH, or pH_i) to drop from 7.3 (normal value) down to 7, and later to 6.5 in more advanced stages of cancer and in metastases the pH_i drops to 6.0 and even 5.7 or lower.[5] Interestingly in various human tumors, especially of epithelial origin or gliomas, the inducible chaperones HSP70 (member of the HSP70 superfamily) and HSP27 (member of the small HSP family) are reported at elevated expression levels, revealing a potential role of HSF1 in carcinogenesis.[3] Furthermore, heat shock (elevated temperature) also causes a significant drop[6] (the pH_i value drops down to 6.0 from a normal value) in mammalian cells, along with activation of HSF1 and overexpression of HSPs. Another experiment presented that cytoplasmic extracts from HeLa cells were incubated with various pH buffers ranged from 5.0 to 7.8. DNA-binding activities of HSF1 were occurred in extracts adjusted to a pH from 5.8 to 6.4, with maximal activation occurring at pH 6.0.[7] Our result proved that purified HSF1 could also form a homotrimer after directly incubated in low pH buffer (pH range from 6.4 to 5.8) (unpublished data), in keeping with the above DNA binding assay result.[7] Thus, all these evidences strongly suggest that an increasing hydrogen ion (H^+) or an acidification of specific amino acid (especially pH value is down to 6.0) could be involved in HSF1 activation, and triggers the translation of HSP genes.

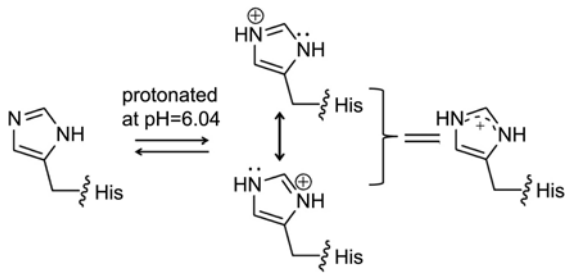

Figure 1. The structures of the imidazole ring of histindine residues in the neutral and cationic (protonated) states.

According to pK_a of the side chains in 20 known amino acids, the imidazole ring of histidine has a pK_a of 6.04,[8] which means that, at physiologically change of pH values, relatively small shifts in pH will protonate most of the imidazole rings in histidine residues. After protonated, the imidazole ring shares two NH bonds, and owns a positive charge which is equally distributed between both nitrogen atoms, thus representing a resonance structure (Figure 1).[9] The positive charge arisen from the protonated imidazole ring could generate the new inter- or intra- molecular forces with the other molecules, thus causing a potential structural change of proteins.[10-12]

Tryptophan(Trp)-Fluorescence spectroscopy (TFS) is a traditional and widely used method for the investigation of protein dynamic structural conformations. By general excitation at 280 nm, most of the emission signals in protein are due to excitation of tryptophan residues.[8] Human HSF1 has three tryptophan residues, and two of which (Trp_{23} and Trp_{37}) are located in HSF1 DNA-binding domain (DBD). TFS has been previously adopted to prove that Trp_{37} residue is involved in an intermolecular π-π stacking interaction which results in heat-induced HSF1 trimerization.[13,14]

In this study, we chose HSF1 DBD as a model to find which histidine residue is important for the low pH dependent activation, because: (1) DBD is the most conserved domain in HSF1 throughout all known organisms,[14] which exhibits various stress-induced DNA-binding activities. (2) Recently, 3D structure of HSF1 DBD by NMR spectroscopy has been solved, which helps us to find the target His residue easily.

In Figure 2(a), heat-induced (at the temperatures of 40 °C and 42 °C) trimerization and disulfide-bond formation of the

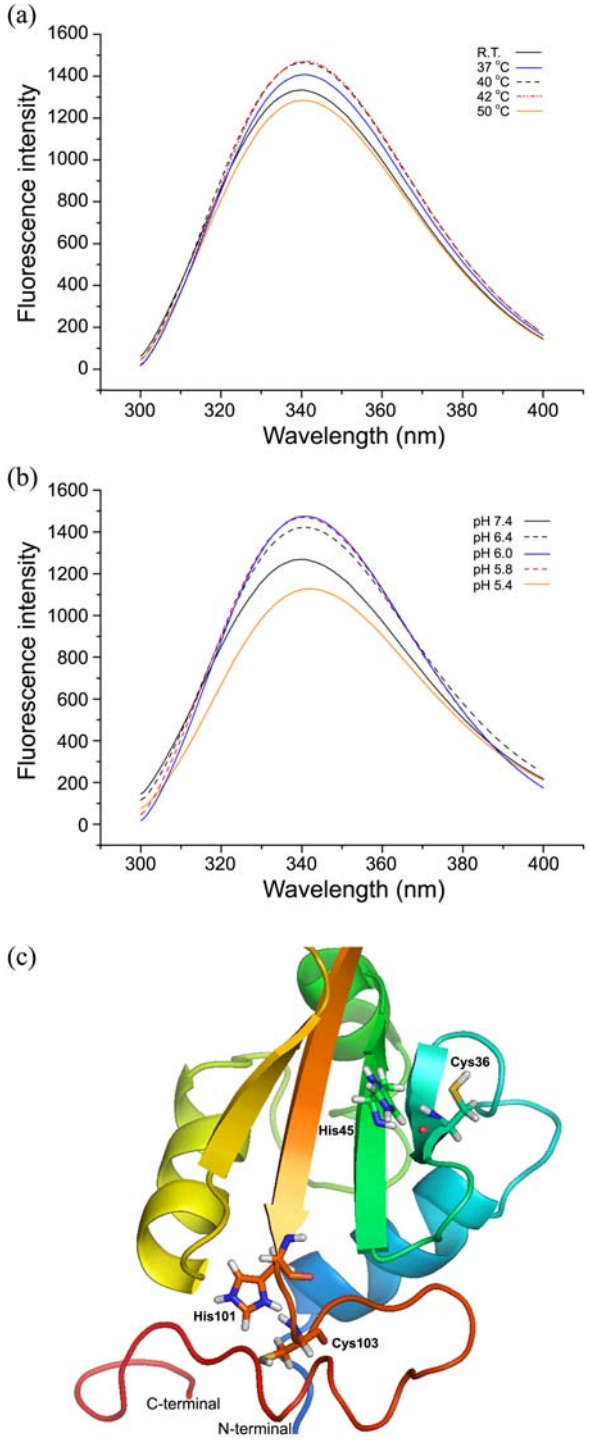

Figure 2. TFS of HSF1 under the different temperatures (a), and pHs (b) conditions. The detail protocols were shown in experimental section. (c) NMR structure of human HSF1 DNA-binding domain (PDB ID: 2ldu) generated by PyMol program. The residues Cys_{36}, His_{45}, His_{101}, and Cys_{103} were labeled.

DNA binding domain (DBD) in human HSF1 lead to an increasing signal in TFS (Figure 2(a)), suggesting a structural change (a more folding state) of HSF1.[13] We also found that HSF1 in low pH buffer exhibited a similar phenomenon like heat induced experiment (Figure 2(b)), with maximal folding state occurring at 6.0 and 5.8 of pH value. Thus, these results

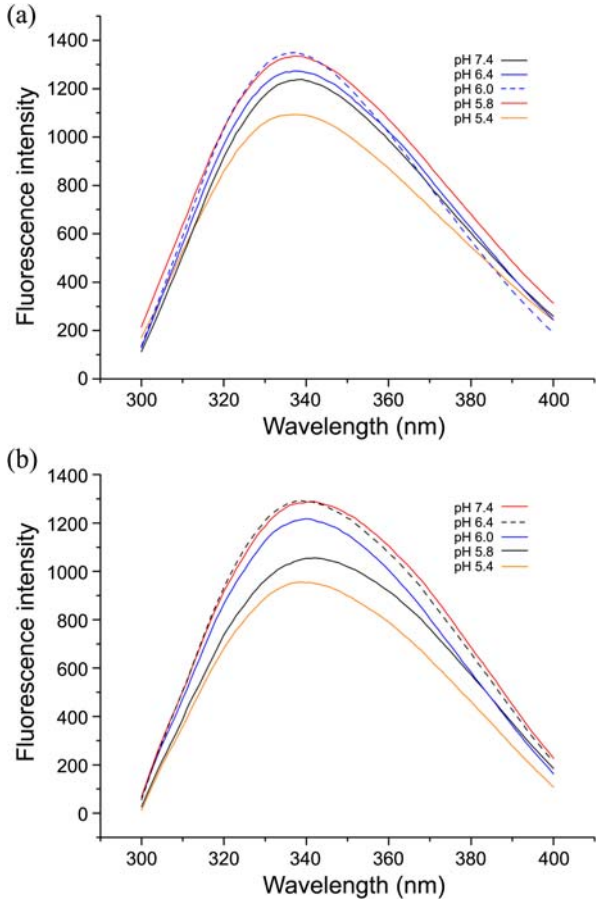

Figure 3. TFS of His45A (a), and His101A (b) DNA-binding domain (DBD) of HSF1 mutants in various pH buffers.

suggest that TFS could be used as a simple tool to seek histidine residues involved in acid-induced HSF1 activation.[13]

Previous studies have suggested that formation of inter-disulfide bond (between Cys_{36} and Cys_{103} residues) is the key step for human HSF1 activation.[15-17] We thus examined which histidine residues near to Cys_{36} or Cys_{103} could influence HSF1 trimerization. Furthermore, the solution structure of human HSF1 DBD (Figure 3) has been solved by NMR spectroscopy, and His_{45} residue, located in the second β-sheet, is spatially next to Cys_{36}, whereas His_{101} also faces the outside of HSF1 DBD, together with Cys_{103}. We thus point mutated two histidine residues in HSF1 DBD to alanine by PCR-mediated, site-directed mutagenesis kit, respectively. Their TFS results (Figures 3(a) and 3(b)) were analyzed following the protocols with the wild-type HSF1 protein (Figure 2(b)).

His_{45}A mutant HSF1-DBD showed the similar tendency, compared with its wild-type one, nevertheless His_{101}A mutant could not be stimulated to a more folding state in pH 6.0 and 5.8 (Figures 2(b), 3(a), and 3(b)), suggesting that the residue His_{101}, but not His_{45}, is important for HSF1 activation. In previous studies, histindine-participant interactions have been found including the intra- or inter- molecular interaction.[10-12] Most of them are related with tryptophan residue to form a specific cation-π force.[18] As reported in a paper,[16] HSF1 has

two tryptophan (Trp_{23} and Trp_{37}) residues in its DNA-binding domain. Trp_{37} is involved in heat-induced HSF1 activation by a specific intermolecular π-π interaction.[13] Point mutation of these two Trp residues did not change the secondary structure by CD spectroscopy.[13] The distances of Trp_{23}-His_{101} (16 Å) and Trp_{37}-His_{101} (18 Å) in DBD were calculated by Discovery Studio Client program. 99% of significant cation-π interactions occur within a distance of 6.0 Å.[19] Thus, we inferred that His_{101} may interact with an unknown Trp residue in HSF1 to form a pH-dependent inter- (but not intra-) molecular cation-π interaction which activate HSF1 trimerization and DNA-binding.

Experimental Section

Protein Preparation. *E. coli* BL21 (DE3) was transformed with pET21b-human HSF1 or its histidine mutants (His_{45}A and His_{101}A). Point mutation of HSF1 was performed with PCR-mediated, site-directed mutagenesis. *E. coli* cells were cultured in LB medium to an OD_{600} of 0.6-0.8, and the targeted proteins were induced by adding 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and culturing for 12 h at 20 °C. The cells were collected by centrifugation and then resuspended in binding buffer containing 20 mM Tris-HCl (pH 8.0), 30 mM imidazole, 200 mM sodium chloride, and 0.5 mM PMSF. After sonication, crude lysate was centrifuged at 13000 rpm for 40 min at 4 °C. Supernatant was loaded onto a HisTrap HP column by FPLC (GE Healthcare), and HSF1 was eluted using an elution buffer containing 250 mM imidazole. HSF1 was further purified using a mono Q HR 5/5 column (GE Healthcare), as described previously.[15] The fractions that were at least 90% pure as determined by Coomassie staining were collected and stored at −80 °C.

Trp-Fluorescence Spectroscopy (TFS). TFS samples (200 μg/mL) were dialyzed in TGE buffer. TFS was performed with a FP-750 spectrometer (JASCO). For the selective excitation of Tryptophan amino acid, a wavelength of 280 nm was used (parameters were setup as $\lambda_{excitation}$ = 280 nm, the range of sample fluorescence emission was 300-400 nm).[13]

Acknowledgments. This study was supported by Pusan National University Grant (2011-2012) for two years. Ming Lu was financially supported by the 2012 Post-Doc. Development Program of Pusan National University.

References

1. Anckar, J.; Sistonen, L. *Annu. Rev. Biochem.* **2011**, *80*, 1089.
2. Åkerfelt, M.; Morimoto, R. I.; Sistonen, L. *Nat. Rev. Mol. Cell Bio.* **2010**, *11*, 545.
3. Dai, C.; Whitesell, L.; Rogers, A. B.; Lindquist, S. *Cell* **2007**, *130*, 1005.
4. Webb, B. A.; Chimenti, M.; Jacobson, M. P.; Barber, D. L. *Nat. Rev. Cancer* **2011**, *11*, 671.
5. McCarty, M. F.; Whitaker, J. *Altern. Med. Rev.* **2010**, *15*, 264.
6. Liu, F. F.; Sherar, M. D.; Hill, R. P. *Radiat. Res.* **1996**, *145*, 144.
7. Mosser, D. D.; Kotzbauer, P. T.; Sarge, K. D.; Morimoto, R. I. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 3748.
8. Lehninger, A. L.; Nelson, D. L.; Cox, M. M. *Principles of Biochemistry*, 2ed.; Worth Publishers: New York, USA, 1993; p 113.
9. Li, S.; Hong, M. *J. Am. Chem. Soc.* **2011**, *133*, 1534.
10. Van, G. M.; Hudson, B. S. *Biophys. Chem*. **1996**, *63*, 17.
11. Okada, A.; Miura, T.; Takeuchi, H. *Biochemistry* **2001**, *40*, 6053.
12. Otomo, K.; Toyama, A.; Miura, T.; Takeuchi, H. *J. Biochem.* **2009**, *145*, 543.
13. Lu, M.; Lee, Y. J.; Park, S. M.; Kang, H. S.; Kang, S. W.; Kim, S.; Park, J.-S. *Biochemistry* **2009**, *48*, 3795.
14. Park, J.-K.; Kim, S.-J. *Bull. Korean Chem. Soc.* **2012**, *33*, 1839.
15. Lu, M.; Kim, H. E.; Li, C. R.; Kim, S.; Kwak, I. J.; Lee, Y. J.; Kim, S. S.; Moon, J. Y.; Kim, C. H.; Kim, D. K.; Kang, H. S.; Park, J.-S. *Biochemistry* **2008**, *47*, 6007.
16. Lu, M.; Sohn, K. J.; Kim, S. W.; Li, C. R.; Kim, S.; Kim, D. K.; Park, J.-S. *Biochem. Biophys. Res. Commun.* **2009**, *385*, 612.
17. Lu, M.; Kim, H.-E.; Li, C.-R.; Hwang, Y.-J.; Kim, S.; Kwak, I.-J.; Lee, Y.-J.; Kim, S.-S.; Kang, S.-W.; Park, N.-G.; Kim, D.-K.; Kang, H. S.; Park, J.-S. *Bull. Korean Chem. Soc.* **2007**, *28*, 1455.
18. Alagona, G.; Ghio, C.; Giolitti, A.; Monti, S. *Theor. Chem. Acc.* **1999**, *101*, 143.
19. Gallivan, J. P. *Proc. Natl. Acad. Sci. USA.* **1999**, *96*, 9459.