Identification of a Key Residue in *Drosophila* Heat Shock Factor-DNA Interaction by Analytical Ultracentrifugation

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Received April 10, 1999

In response to elevated temperature and a variety of chemical treatments, all organisms rapidly produce heat shock proteins, which protect the structure and activity of proteins from denaturation under environmental stresses.[1] In eukaryotes, the regulation of the heat shock response is mediated by a preexisting transcription factor, heat shock factor (HSF), which binds to a conserved regulatory DNA sequence, heat shock element (HSE) with high affinity and specificity.[2] HSE is a conserved sequence located upstream of all heat shock genes containing a series of small GAA boxes, each with the sequence NGAAN, where N is any nucleotide.[3] The activation of HSF involves heat shock induced trimerization, binding to its cognate DNA sites, and the acquisition of transcriptional competence. The trimerization of purified *Drosophila* HSF was shown to be sensitive directly to heat, oxidation and pH.[4,5] Structural studies using X-ray[6] and NMR[7] indicated that the DNA-binding domain of HSF forms a compact structure resembling the helix-turn-helix DNA-binding motif where helix 3 serves as a putative DNA recognition helix. The DNA binding helix of *Drosophila* HSF start with M97 and end with Y107 (97-MASFIRQLNMY-107), but no structural data on HSF-DNA complex or biophysical informations on the key residues involved in the complex formation is available.

The effects of HSE DNA mutation on the affinity of HSF-HSE interaction using 131 amino acid wild-type HSF DNA binding domain (DBD, dHSF(33-163)) were studied by analytical ultracentrifugation previously.[8] From the study, it was shown the wild-type DBD can bind as a monomer with 1 : 1 stoichiometry to a synthetic DNA containing a single NGAAN sequence and there is about 20 to 300 fold difference in DNA binding affinity between wild-type and mutant HSE DNA depending on salt (0.05 M to 0.15 M) and temperature (2 °C to 18 °C) studied. In this present study, we extended our previous study and have generated two mutant HSF DBDs using a modified enzymatic reverse polymerase chain reaction technique[9,10] where arginine 102 and asparagine 105 on the *Drosophila* HSF DNA recognition helix were replaced to alanines (R102A & N105A) and studied the effect of mutation on the protein-DNA interactions. To establish the stoichiometry of binding first, the oligomeric states of mutant DBDs and the homogeneity of the double-stranded, 17-bp synthetic DNAs were evaluated by using Beckman XL-A analytical ultracentrifuge. The affinity of binding for the mutant and wild-type DBDs with wild-type 17-bp HSE DNA was then determined by multi-wavelength scan technique[8,11] using analytical ultracentrifugation.

The DNA binding specificity and the monomeric nature of wild-type DBD (dHSF(33-163)) were demonstrated previously.[8] Figures 1A and 1B shows the sedimentation equilibrium concentration distributions of R102A and N105A mutant DBDs at 230 nm fit by a thermodynamically ideal monomeric (solid line) and dimeric (dotted line) species with molecular masses of monomers for R102A and N105A mutants of 15,174 and 15,216 Daltons, respectively calculated from the amino acid sequences. The residual plots for the given models are also shown on the top of the figures. The deviation from the dimeric models and the good quality of monomeric fit demonstrate that these two mutant polypeptides exist as monomers in solution like wild-type DBD. Figure 1C illustrates the concentration distribution of a 17-bp, double-stranded oligonucleotide, 5'-GGGCACA-GAAAGCCGCC-3' fit with a molecular mass, calculated from the base pair composition, of 10,474 Daltons. The data demonstrate that the HSE is a 17-bp double-stranded DNA with no detectable single-stranded DNA contamination.

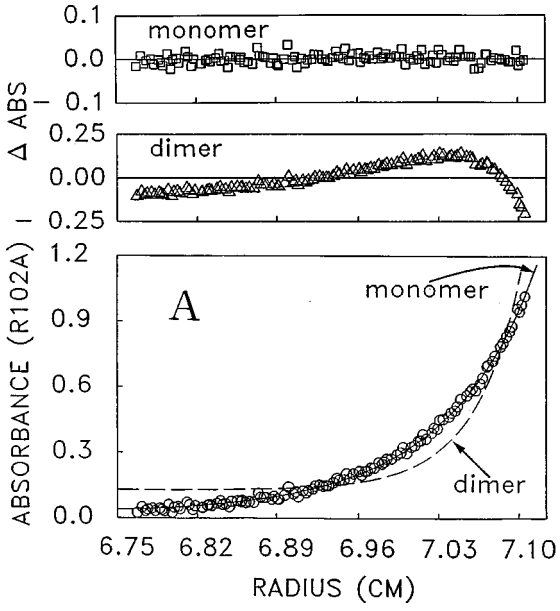

Figure 1A. (top) Distributions of the residuals of the dHSF (33-163) R102A for the monomeric and dimeric models as a function of radial positions. (bottom) A distribution of absorbance of the dHSF (33-163) R102A at 298.15 K, 230 nm and 30,000 rpm. The solid line represent the monomeric fitting line with a calculated molecular mass of 15,174 Da and the dotted line for the theoretical dimeric fitting line.

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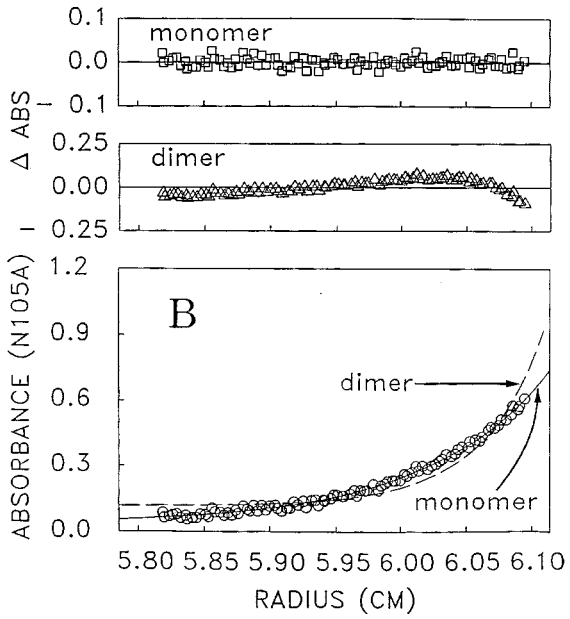

Figure 1B. (top) Distributions of the residuals of the dHSF (33-163) N105A for the monomeric and dimeric models as a function of radial positions. (bottom) A distribution of absorbance of the dHSF (33-163) N105A at 298.15 K, 230 nm and 30,000 rpm. The solid line represent the monomeric fitting line with a calculated molecular mass of 15,216 Da and the dotted line for the theoretical dimeric fitting line.

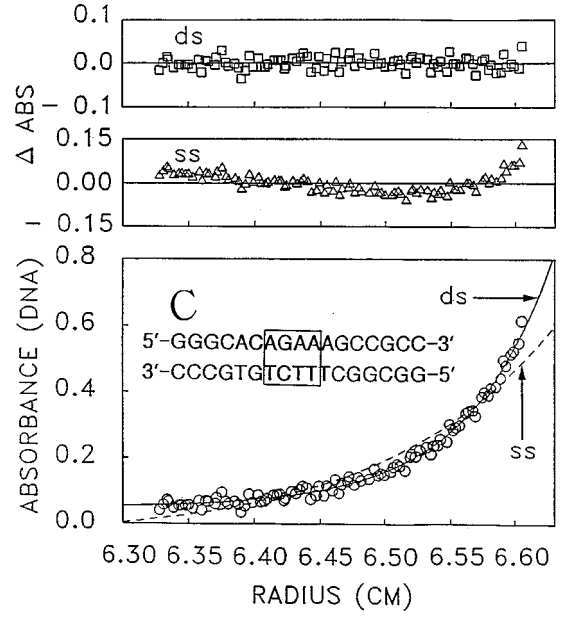

Figure 1C. (top) A distribution of the residuals of the 17-bp DNA for the single and double stranded models as a function of radial positions. (bottom) Distributions of absorbance of the 17-bp DNA at 298.15 K, 230 nm and 30,000 rpm. The calculated molecular weight of 10,474 Da for 17-bp DNA was used for the double-stranded model fitting and 5,237 Da was used for the single-stranded model fitting. (Inset) The sequence of 17-bp HSE DNA.

After establishing that the mutant DBDs, R102A and N105A, are monomers in solution and that the wild-type HSE is a 17-bp double-stranded DNA, we have calculated the differences in the binding affinity due to the single-point

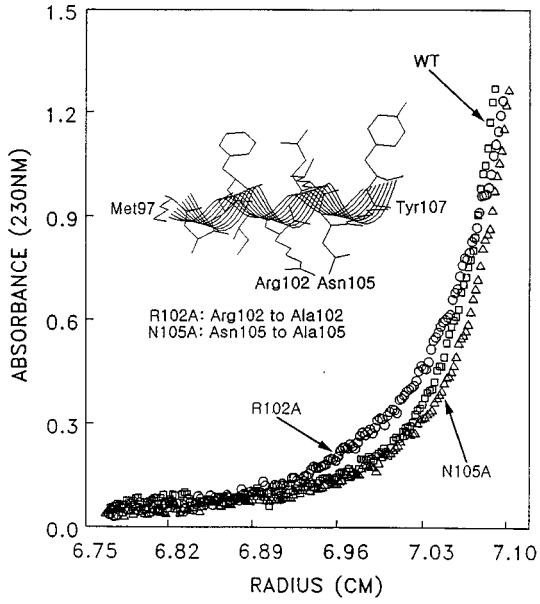

Figure 2. Equilibrium distributions of the absorbancies of mixtures of 17-bp HSE DNA with wild-type dHSF (33-163) (squares), R102A (circles) and N105A (triangles), respectively, in a 2 to 1 DNA to polypeptide molar ratio at equilibrium at 30,000 rpm at 25 °C in pH 6.3, 10 mM potassium phosphate buffer containing 0.1 M KCl and 0.1 mM EDTA. A ribbon diagram of helix 3 showing the locations of the two residues mutated to alanines. The data were collected at wavelengths 230 nm to 240 nm with a 2 nm increments, but, in order to avoid confusion, only the data at 230 nm is shown. The corresponding polypeptides are indicated with arrows.

mutation. Figure 2 shows scans of the equilibrium distributions of the polypeptide-DNA complexes of the wild-type DBD (square), R102A (circle) and N105A (triangle) measured at 230 nm at ultracentrifugal equilibrium in 10 mM potassium phosphate buffer containing 0.1M KCl at 25 °C at pH 6.3. The same concentrations of polypeptide and DNA were loaded at each centrifuge cell to facilitate the comparison. On the basis of observable differences between the concentration on gradients, it is obvious that the wild-type and N105A mutant polypeptides form stronger HSE-polypeptide complexes than the HSE-R102A complex. However, from the concentration gradients of the complexes, the N105A mutant polypeptide seems to forms slightly less but as tight complex as the HSE and wild-type DBD complex.

In order to obtain more quantitative differences in the values of the association constants (K_a) for the HSE-polypeptides interactions, the data were analyzed by using previously described multiwavelength scan analysis method.[8,11] In Table 1, the results of the analysis for the mutant and the wild-type DBD interactions with 17-bp HSE DNA are summarized. Based on the data, the mutation of arginine 102 to alanine 102 decreased the binding affinity about 42-fold weaker than does the wild-type DBD indicating the critical role of the R102 residue in HSF-HSE interaction. This difference is reflected in a $\Delta\Delta G^o$ value of 2.38 kcal mol^{-1} at 25 °C, and is caused by a single residue substitution (R102 → A102) in the mutant DBD (Table 1). The value of -ΔG^o for

Table 1. A Summary of the binding parameters for wild-type and mutant polypeptide-HSE interactions in 10 mM potassium phosphate buffer at pH 6.3 containing 0.1 M KCl at 25 °C

	K_a	ln K_a*	$-\Delta G^0$ (kcal mol^{-1})	$\Delta\Delta G^0$ (kcal mol^{-1})
wild-type	1.38×10^6	14.42 ± 0.14	8.54	0
R102A	3.29×10^4	10.40 ± 0.10	6.16	2.38
N105A	1.13×10^6	13.94 ± 0.13	8.26	0.28

*K_a: association constant; ln K_a: natural log of association constant; ΔG^0: free energy of binding; $\Delta\Delta G^0$: differences in free energy of binding (wild-type-mutant).

the binding of wild-type DBD (8.54 kcal mol^{-1}) and N105A mutant DBD (8.26 kcal mol^{-1}) with the $\Delta\Delta G^o$ of 0.28 kcal mol^{-1} support the minimal role of N105A residue in the binding process. The minimal role of N105 in the process is rather unexpected considering that N105 is highly conserved among the HSF family.[6,7]

In summary, we have shown the wild-type and mutant DBDs exist as monomers in solution by means of equilibrium analytical ultracentrifugation and have determined the equilibrium binding constants for the interactions between the 131 amino acid purified mutant and wild-type polypeptides and 17-bp HSE DNA. By appropriate analysis of the data, we have been able to calculate the values of the association constants and the free energies of binding for the interactions. Our data suggest that the highly conserved R102 plays a major role in HSE recognition but the N105 residue plays much lesser role in the DNA binding process. A detailed analysis on the thermodynamic properties of mutant polypeptides and HSE binding process is under investigation.

Acknowledgment. We thank the National Instrumentation Center for Environmental Management (NICEM) for allowing us to use Beckman XL-A analytical ultracentrifuge. We also thank Dr. Carl Wu for supporting some materials and encouragements. This work was supported by the Korea Science and Engineering Foundation (KOSEF) project number 961-0303-025-02 with a partial support by KOSEF project number 95-0403-01-3.

References

1. In *The Biology of Heat Shock Protein and Molecular Chaperones*; Morimoto, R. I., Tissieres, A., Georgopoulos, C., Eds.; Cold Spring Harbor Press: New York, 1994.
2. Wu, C. *Ann. Rev. Cell & Dev. Biol*. **1995**, *11*, 441.
3. Perisic, O.; Xiao, H.; Lis, J. T. *Cell* **1989**, *59*, 797.
4. Zhong, M.; Oresz, A.; Wu, C. *Mol. Cell* **1998**, *2*, 101.
5. Zhong, M.; Kim, S.-J.; Wu, C. *J. Biol. Chem.* **1999**, *274*, 3135.
6. Harrison, C. J.; Bohm, A. A.; Nelson, H. C. M. *Science* **1994**, *263*, 224.
7. Vuister, C. W.; Kim, S.-J.; Oresz, A.; Marquardt, J.; Wu, C.; Bax, A. *Nat. Struct. Biol.* **1994**, *1*, 605.
8. Kim, S.-J.; Tsukiyama, T.; Lewis, M. S.; Wu, C. *Protein Sci*. **1994**, *3*, 1040.
9. Stemmer, W. P. C.; Morris, S. K. *BioTechniques* **1992**, *13*, 215.
10. Orosz, A.; Wisniewski, J.; Wu, C. *Mol Cell Biol.* **1996**, *16*, 7018.
11. Lewis, M. S.; Shrager, R. I.; Kim, S.-J. In *Modern Analytical Ultracentrifugation: Acquisition and Interpretation of Data for Biological and Synthetic Polymer Systems*; Schuster T. M., Laue T. M., Eds.; Birkhäuser Inc.: Boston, U.S.A., 1994; p 94.