Specific DNA Binding by the Homeodomain Nkx2.5(C56S): Detection of Impaired DNA or Unfolded Protein by Isothermal Titration Calorimetry

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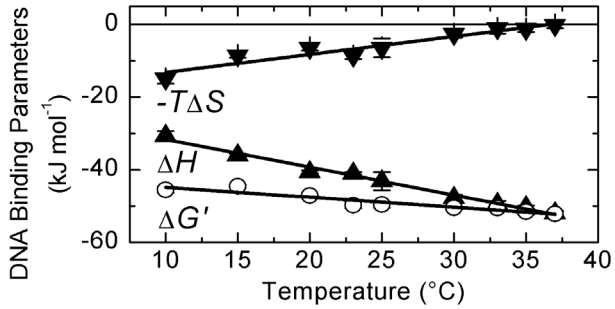
Titrations of specific 18 bp duplex DNA with the cardiac-specific homeodomain Nkx2.5(C56S)[a] have utilized an ultra sensitive isothermal titration calorimeter (ITC); [1,2]. As the free DNA nears depletion, we observe large apparent decreases in the binding enthalpy when the DNA is impaired or when the temperature is sufficiently high to produce some unfolding of the free protein. Either effect can be attributed to refolding of the biopolymer that occurs as a result of stabilization due to the large favorable change in free energy on the homeodomain binding to DNA (-49.4 kJ/mol at 298 K). In either case, thermodynamic parameters obtained in such ITC experiments are unreliable. By using a lower temperature (85 vs. 95 °C) during the annealing of complementary DNA strands, damage of the 18 bp duplex DNA (T_m = 72 °C) is avoided, and titrations with the homeodomain are normal at temperatures from 10 to 40 °C when >95 % of the protein is folded. Under the latter conditions, the heat capacity plot is linear with a ΔC_p value of -0.80 ± 0.03 kJ K^{-1} mol^{-1} which is more negative than that calculated from the burial of solvent accessible surface areas (-0.64 ± 0.05 kJ K^{-1} mol^{-1}), consistent with water structures being at the protein-DNA interfaces.



[a] The Nkx2.5 protein used in our studies is a 79-residue segment encompassing the homeodomain with the oxidizable C56 replaced by isosteric Ser.

[1] E. Fodor, J.W. Mack, J.-S. Maeng, J.-H. Ju, H.S. Lee, J.M. Gruschus, J.A. Ferretti, and A. Ginsburg, *Biochemistry*, **44**, 12480 (2005).
[2] E. Fodor, and A. Ginsburg, *PROTEINS: Structure, Function, and Bioinformatics* (2006, *in press*).