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The thermodynamic effect of a designed disulphide bond on the molecular surface of cold shock protein from *Thermus thermophilus HB8.*

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It is widely believed that intramolecular disulphide (SS) bonds of protein molecules stabilize the three-dimensional structure by decreasing the chain entropy of the denatured state. Many of the experimental results of the observed effect on the entropy change of denaturation by introducing or deleting SS bond, however, cannot be explained simply by the calculated entropy change of the denatured state, but indicates the effect on the thermodynamic properties of the native state. In order to clarify the effect in detail, the thermodynamic stability and the molecular function of the mutant of cold shock protein from *Thermus thermophilus HB8*, A11C/T40C, with a SS bond introduced on the molecular surface were evaluated. The stability was evaluated by highly precise differential scanning calorimetry (DSC) and the molecular interaction with single stranded DNA molecules (ssDNA) was by isothermal titration calorimetry (ITC). The wild type and the mutant proteins were found to show monomeric and highly reversible 2-state-like thermal transitions under the acidic pH condition and to be stabilized by the entropic effect almost the same as that from the contribution from the entropic reduction of the denatured state by the SS bond. The enthalpic destabilization effect, about 10 kJ/mol, was also observed suggesting the small effect onthe native conformation. Although the mutant can bind ssDNA strongly, the binding constant was found to be lower than that of the wild type and to be recovered by reduction of the SS bond, suggesting the effect of the SS bond formation on the conformation of the native state.