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# Effect of the polypeptide binding on the thermodynamic stability of the substrate binding domain of the DnaK chaperone

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**Abstract**

The effect of polypeptide binding on the stability of the substrate binding domain of the molecular chaperone DnaK has been studied by thermodynamic analysis. The calorimetric scan of the fragment of the substrate binding domain DnaK384–638, consisting of a β-domain and an α-helical lid, showed two transitions centered at 56.2 and 76.0 °C. On the other hand, the thermal unfolding of the shorter fragment DnaK386–561, which lacks half of the α-helical lid, exhibited a single transition at 57.0 °C. Therefore, the transition of DnaK384–638 at 56.2 °C is mainly attributed to the unfolding of the β-domain. The calorimetric scan of DnaK384–638D526N showed that the unfolding of the β-domain was composed of two transitions. The polypeptide bound DnaK384–638 exhibited a symmetrical DSC peak at 58.6 °C, indicating that the substrate binding shifts the β-domain toward a single cooperative unit. A low concentration of GdnHCl (<1.0 M) induced a conformational change in the β-domain of DnaK384–638 without changes in the secondary structure. While the thermal unfolding of the β-domain of DnaK384–638 was composed of two transitions in the presence of GdnHCl, the β-domain of the substrate bound DnaK384–638 exhibited a single symmetrical DSC peak in the same condition. All together, our results indicate that complex between DnaK384–638 and substrate forms a rigid conformation in the β-domain.


*Keywords:* Molecular chaperone; DnaK; Substrate binding domain; DSC; Thermal unfolding; Limited proteolysis

## 1. Introduction

The *Escherichia coli* molecular chaperone DnaK, a member of the HSP70 proteins family, facilitates the folding of polypeptides and prevents their aggregation [1–3]. DnaK consists of an N-terminal ATPase domain and a C-terminal substrate binding domain (SBD). Substrate binding and release is allosterically controlled by adenine nucleotides [4]: When ATP is bound to the ATPase domain, the substrate binding domain of DnaK (DnaK's SBD) binds substrate weakly (low affinity state), whereas ATP hydrolysis brings about a conformational change in the ATPase domain which in turn converts the SBD to a high affinity state. The 3D structures of several fragments of SBD have been solved [5–10] as shown in Fig. 1. The structure of a peptide–DnaK (residues 384–607) complex has been solved by X-ray crystallography [5] and shows that the SBD consists of a β-domain (393–501, consisting of strand β1−8) and an α-helical lid (509–607, consisting of helices A–E). The remaining C-terminal domain from residue 608 to 638 comprises random coil [10]. The deletion of this region affects the ATPase binding, suggesting that this region interacts to the ATPase domain to create a closed-to-open equilibrium [11]. Substrate binding occurs by a dynamic mechanism in a two-layered closing device involving the independent action of an α-helical lid and an arch formed by M404 and A429. The SBD is in equilibrium between the open and closed conformations, and the open conformation is largely populated in the ATP bound states [4]. The NMR structure of SBD fragment DnaK386–561 [6], in which helices C–E were deleted,

*Abbreviations:* SBD, substrate binding domain; GdnHCl, guanidine hydrochloride; RCMLA, reduced and carboxylmethylated α-lactalbumin

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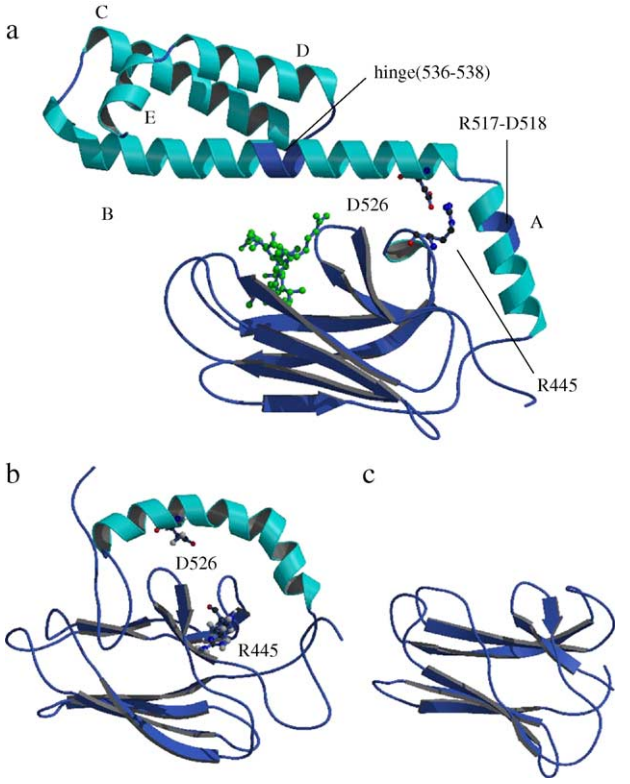


Fig. 1. 3D structure of the various DnaK's SBD fragments. (a) DnaK389–607 complexed with a model peptide (PDB code 1DKZ [5]). R445 and D526, which form a salt-bridge [8], are shown in the ball-and-stick form. The substrate model peptide is shown in green in the ball-and-stick form. (b) DnaK386–561 (PDB code 1BPR [6]). R445 and D526 are shown in the ball-and-stick form. (c) DnaK393–507 (PDB code 1DG4 [7]). All figures were drawn with MOLSCRIPT [33] and Raster 3D [34].

suggests that the lid pivots on the β-domain during the opening of the SBD (Fig. 1b).

The interaction between DnaK and its substrate has been investigated in detail [12–19]. The NMR study of a lidless SBD fragment DnaK393–507 revealed that the binding of a substrate induces conformational changes in the β-domain in absence of any interaction with the α-helical lid [7]. The kinetic for the substrate binding of DnaK in the ADP bound states is slow because of a large activation energy barrier [13]. DnaK384–638, a fragment of SBD, binds substrates with high affinity in a similar manner to DnaK in the ADP bound state [14,15,19], indicating that the majority of molecule is in the closed conformation in the solution. The thermal unfolding of the full-length DnaK [20,21], a fragment of DnaK's SBD (DnaK387–638) [21] and a fragment of human HSP70's SBD [22] has been described previously. These studies revealed that the full-length DnaK and the fragments of SBD exhibited multiple transitions in the thermal unfolding. In this study, we have studied the effect of the polypeptide binding on the thermal unfolding of DnaK's SBD fragments in order to gain insight into the effect of substrate binding on its conformation. We found that the substrate binding significantly changes the thermal unfolding transition of DnaK's SBD through the DSC measurement.

## 2. Materials and methods

### 2.1. Materials

The expression vector pDKC carrying DnaK384–638 with a 6xHis tag at the N-terminus was kindly provided by Dr. W.F. Burkholder (MIT) and Prof. M.E. Gottesman (Columbia University). The expression vector for DnaK386–561 and DnaK386–507 was constructed by PCR amplification and cloned into a pRSETA vector (Invitrogen (Carlsbad, CA)). The cloned gene fragments were sequenced to ensure that no mistakes had been introduced during the amplification process. DnaK's SBD mutants were constructed using the quickchange site-directed mutagenesis kit following the manufacturer instructions (Stratagene, La Jolla, CA). Recombinant proteins were expressed as previously described [19], purified by affinity chromatography using a NiNTA resin column following the manufacturer's instructions (Qiagen Inc., Valencia, CA). The reduced and carboxylmethylated α-lactalbumin (RCMLA) was obtained from Sigma Chemical Co. (St. Louis, MO). 10 mM Tris pH 7.0 was used as the solvent for the all experiments. Self-association of the fragment of the substrate binding domain of DnaK has been reported in previous studies [14,23] perhaps through the C-terminal random coil region [11]. However, the oligomerization of DnaK's SBD was not monitored by native gel electrophoresis in our experimental condition (data not shown). This would be due to the difference in the solvent condition and the protein concentration.

### 2.2. DSC

Calorimetric measurements were performed on a Nano-DSC II Model 6100 (Calorimetry Science Co., UT, USA). Most experiments were done at a scan rate of 1.0 °C/min and a protein concentration of 0.7–1.5 mg/ml. All data analyses, i.e. baseline subtraction, concentration normalization and deconvolution, were performed using the software provided by the manufacturer (Calorimetry Science Co., UT, USA).

The van't Hoff enthalpy ($\Delta H_{vH}$) is obtained with the standard formula,

$$\Delta H_{vH} = 4\ R\ T_m^2 C_{p,\ max}\ /\Delta H_{cal}$$

where $C_{p,max}$ is the maximum of the excess heat capacity function, $T_m$ is the transition temperature defined as the temperature location of $C_{p,max}$ and $R$ is the gas constant.

### 2.3. Limited proteolysis

Limited proteolysis was performed as described previously [24]. N-terminal sequence analysis was carried out on the peptide samples isolated by blotting from a gel using an Applied Biosystems (Foster City, CA) protein sequencer (model 476A) equipped with an on-line analyzer