Structure of the Molecular Chaperone ClpB from *Thermus thermophilus*

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Introduction

The bacterial molecular chaperone ClpB and its eukaryotic homologs Hsp101 and Hsp104 form large ring-like structures and are essential proteins of the heat-shock response. However, unlike more "conventional" chaperones, such as GroEL/Hsp60 and DnaK/Hsp70, members of the ClpB/Hsp104 family (ClpB/Hsp104) do not promote the "forward" folding or prevent the aggregation of proteins. Instead, ClpB/Hsp104 has the remarkable ability to rescue stress-damaged proteins from an aggregated state. The full recovery of these proteins requires the assistance of the cognate DnaK/Hsp70 chaperone system. However, the mechanism by which ClpB/Hsp104 cooperates with the DnaK/Hsp70 chaperone system remains unknown.

To understand the structure-function relationship and to determine the mechanism for protein disaggregation by the ClpB/Hsp104 molecular chaperones, we have solved the 3.0-Å-resolution crystal structure of *Thermus thermophilus* ClpB (*T*ClpB) in complex with adenosine 5′-(β,γ-imido)triphosphate (AMPPNP) [1].

Methods and Materials

*T*ClpB was purified and crystallized as previously described [2]. The crystals belonged to the primitive orthorhombic space group $P2_12_12_1$, with unit cell dimensions a = 109.2 Å, b = 139.6 Å, c = 213.0 Å and $\alpha = \beta = \gamma = 90°$. The large unit cell and the weak diffraction of our crystals required the use of the high-brilliance synchrotron radiation light source provided by Structural Biology Center (SBC) beamline 19-ID at the APS. Data were collected on the 3000 × 3000 APS-1 charge-coupled device (CCD) detector. Since our crystals were susceptible to severe radiation damage, the large size and fast readout of this detector was crucial for data collection. The structure of *T*ClpB was determined by the multiwavelength anomalous dispersion (MAD) technique using seleno-methionine substituted protein [1].

Results and Discussion

Our crystal structure reveals that *T*ClpB is a multidomain protein consisting of an N-terminal domain, the D1-large domain or NBD1, the D1-small domain that is composed of the ClpB/Hsp104-linker, the D2-large domain or NBD2, and the D2-small domain (Fig. 1). There are three independent representations of full-length *T*ClpB in the crystallographic asymmetric unit. The three molecules are related approximately by a 60° rotation and a 1/6 translation along the crystallographic twofold screw axis parallel to a. This arrangement of molecules gives rise to a helical assembly that extends throughout the crystal and forms a hexameric ring structure when viewed in projection (data not shown). The most remarkable structural feature is the 85-Å-long, leucine-rich coiled-coil, which consists of the ClpB/Hsp104-linker and resembles in structure the shape of a two-bladed propeller. The long coiled-coil is located on the outside of the *T*ClpB hexamer as determined by electron cryomicroscopy in conjunction with single-particle reconstruction techniques [1]. Most interestingly,

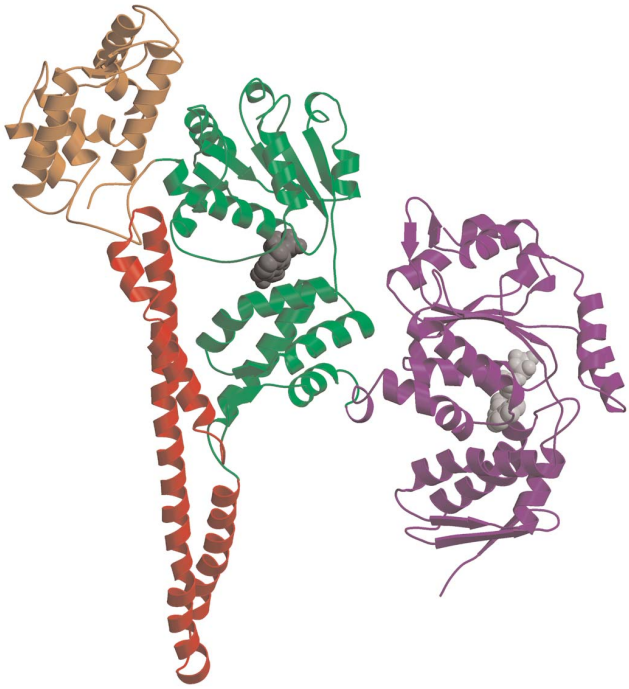

FIG. 1. Ribbon drawing of ***Thermus thermophilus*** *ClpB in the AMPPNP-bound state. The N-terminal domain is gold, the D1-domain is green, the ClpB/Hsp104-linker is red, and the D2-domain is purple. AMPPNP is depicted as a CPK model and is gray.*

a superimposition of the three *T*ClpB representations reveals that the ClpB/Hsp104-linker is mobile. The tip of the long coiled-coil swings by up to 17.3 Å when the three molecules are superimposed through the Cα atoms of NBD2 (data not shown). The location, unusual shape, and mobility of the long coiled-coil suggest that the ClpB/Hsp104-linker may function as a "molecular crowbar" that dissociates large aggregates. By using a combination of structure-based targeted mutagenesis and sulfhydryl cross-linking studies, we have confirmed that the position and motion of the ClpB/Hsp104-linker are critical for chaperone function [1]. We have proposed a mechanism by which the ClpB/Hsp104-linker pulls apart protein aggregates that are bound between the coiled-coil of neighboring *T*ClpB subunits [1]. However, the conformational changes that must occur in ClpB upon ATP binding and hydrolysis have not yet been determined and require further structural, biochemical, and biophysical studies.

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References

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