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Crystal structures of ClpP in complex with acyldepsipeptide antibiotics

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Introduction

Clp-family proteins are prototypes for studying the mechanism of ATP-dependent proteases because the proteolytic activity of the ClpP core is tightly regulated by activating Clp-ATPases. Nonetheless, the proteolytic activation mechanism has remained elusive because of the lack of a complex structure. Acyldepsipeptides (ADEPs), a recently discovered class of antibiotics, activate and disregulate ClpP [1]. Here we have elucidated the structural changes underlying the ClpP activation process by ADEPs. We present the structures of *Bacillus subtilis* ClpP (*Bs*ClpP) alone and in complex with ADEP1 and ADEP2. The structures show the closed- to open-gate transition of the ClpP N-terminal segments upon activation as well as conformational changes restricted to the upper portion of ClpP. The direction of the conformational movement and the hydrophobic clustering that stabilizes the closed structure are markedly different from those of other ATP-dependent proteases, providing unprecedented insights into the activation of ClpP.

Methods

Crystallization was performed using the hanging-drop vapor-diffusion method. We obtained the crystals of free *Bs*ClpP and generated crystals of *Bs*ClpP-ADEP1,2 complex through both soaking and co-crystallization. For the cryo-experiment, crystals were transferred to reservoir solution containing ~25% (w/v) glycerol. We processed the diffraction data with the program HKL2000.

The initial *Bs*ClpP structure was determined by the molecular replacement method using the previously reported structure of *E. coli* ClpP (PDB: 1YG6) as a search model. We mutated the different sequences between *Bs*ClpP and *E. coli* ClpP and refined the structure. The phases of *Bs*ClpP-ADEP1 and *Bs*ClpP-ADEP2 were also obtained by molecular replacement using the refined *Bs*ClpP structure. We refined the models and determined positions of ADEPs using an Fo-Fc difference Fourier map contoured at 2.7σ. We fit the structure of acyldepsipeptides derivative into the map and then modified it to ADEP1 or ADEP2, and refined the models as described above. Program MOLREP, O, COOT, CNS were used to perform the above structure determination and refinement steps.

Results

The structures of free *Bs*ClpP were determined at 2.4 and 3.0Å resolution in two different crystal forms, and those of *Bs*ClpP-ADEP1 and *Bs*ClpP-ADEP2 complexes were also determined at 2.0 and 2.6Å resolution, respectively. Each ClpP tetradecamer is complexed with 14 ADEP molecules in a 1:1 stoichiometry, and the antibiotics are located on the apical and distal surfaces of both ClpP heptameric rings, in cavities formed by two adjacent ClpP monomers (Fig. 1a,b). The 14 ADEP-binding sites in ClpP are deep invaginations in the enzyme surface and contain many hydrophobic residues.

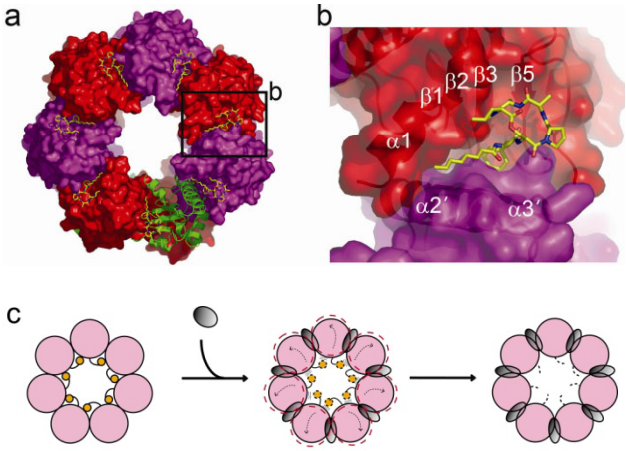

Figure 1. Structure of the *Bs*ClpP-ADEP1 complex and the activation model (a) *Bs*ClpP-ADEP1 complex viewed along a seven-fold molecular symmetry axis (b) Close-up view of an ADEP-binding site boxed in panel a (c) A proposed model for activation of ClpP.

Through superposition of free and ADEP-complexed *Bs*ClpP structures, we found this activator triggers outwards movement of individual subunits of the ClpP body. This conformational change weakens an interaction between the N-terminal segment and protein body of the neighbour subunit in pore region and enhances the pore size of ClpP. (Fig 1c) These ADEP activator-induced close-to-open-gate transition of N-terminal segments provide unprecedented insights into the activation mechanism of ClpP. Recently, We reported these structural insights with another structural analysis, electron microscopy data and supporting additional biochemical experiments. [1]

References

[1] H.B. Brötz-Oesterhelt et al., Nature Med. 11, 1082 (2005)
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