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Crystal structure of zinc binding domain of ClpX in complex with SspB-tail

Eun Young PARK, Byung-Gil LEE and Hyun Kyu SONG*
School of Life Sciences and Biotechnology, Korea University, Anam-Dong
Seongbuk-Gu, Seoul 136-701, South Korea

Introduction

The degradation of ssrA-tagged proteins in the bacterial cytosol is carried out by the proteolytic machine ClpXP, and is markedly stimulated by the adaptor protein SspB. It has previously been reported that the N-terminal zinc-binding domain of ClpX (ZBD) is involved in complex formation with the SspB-tail (XB). In an effort to better understand the recognition of SspB by ClpX and the delivery mechanism of ssrA-tagged substrates to ClpXP ATP-dependant proteases, we have determined the crystal structure of the *E. coli* ZBD of ClpX alone at 1.5, 2.0 and 2.5 Å resolution in each different crystalline lattice and also in complex with the XB peptide at 1.6 Å resolution [1]. The ZBD structure shows a fold similar to treble clef zinc-finger proteins. The XB peptide forms an anti-parallel β-sheet with two β-strands of the ZBD and the structure shows that there are two independent XB binding sites in ZBD. This contrasts with the reported stoichiometry of XB peptide per ZBD dimer obtained from biochemical techniques [2]. Further biochemical analyses using isothermal titration calorimetry (ITC) and activity assays of SspB-ClpX fusion protein shed light on the delivery mechanism of target proteins to the prokaryotic degradation machine.

Materials and methods

The purified ZBD was concentrated to 17 mg/ml in 50 mM Tris-HCl, pH 7.7, 100 mM NaCl and 2 mM β-mercaptoethanol. Crystallization was performed by the hanging-drop vapor diffusion method. Three different crystal forms of free ZBD were obtained. The reservoir solution for the tetragonal crystal consisted of 100 mM tri-sodium citrate, pH 5.6, 2 % ethyleneimine polymer and 500 mM NaCl. The reservoir solution for the orthorhombic form consisted of 100 mM HEPES, pH 7.5, 200 mM $CaCl_2$ and 27–30 % PEG400. Using selenomethionine-derivatized C43M mutant, a new hexagonal crystal form was obtained under crystallization conditions utilizing 100 mM sodium acetate, pH 4.6, 200 mM Li_2SO_4 and 8–12 % 2-propanol. For ZBD-XB complex crystallization, a 10-fold molar excess of solid XB peptide was added and mixture incubated for 30 min at 4 ºC. The complex was crystallized within 10 days over a reservoir of 1.6 M tri-sodium citrate, pH 6.5. The native diffraction data using orthorhombic crystals of free ZBD and ZBD-XB complex, and MAD data using a hexagonal crystal of free C43M mutant were collected on a CCD detector at the NW12 beamline of Photon Factory. All diffraction data were processed and scaled using the HKL2000 software package.

One possible selenium site in the asymmetric unit of the hexagonal crystal form was located with SOLVE. The electron density was of sufficient quality to identify and build a long C-terminal α-helix and a zinc binding site. The model was rebuilt with the program O. The protein model was refined with CNS. Phases of the other crystal forms were obtained by molecular replacement with the program PHASER using refined monomeric ZBD as a search model. The 2-fold non-crystallographic symmetry was maintained with tight restraint during the early stages of refinement, but was relaxed in the final rounds. Phases of the complex crystal were also obtained by molecular replacement using refined model of free ZBD. The positions of the XB peptide were clearly determined using a model-phased difference Fourier map contoured at 3.0 σ. Refinement of the models was also performed as described above.

Results

In the complex structure, the XB peptide binds in a β-strand conformation to a groove formed by strand β1 and helix α1 (Figure 1). We were able to build the last six residues (ALRVVK) of the 8 residue XB peptide. Five of these six residues appear to form the key region in determining the specificity of the interactions with ZBD.

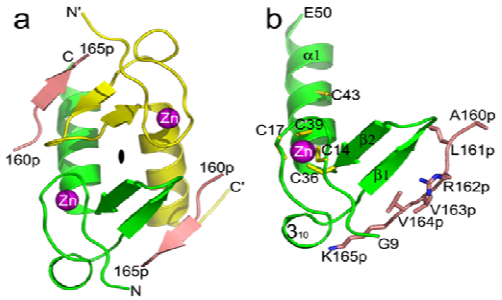

Figure 1. Structure of the ZBD-XB complex. (a) Ribbon diagram showing the dimeric ZBD-XB complex structure. Each monomer in the ZBD is colored green and yellow. The bound XB peptide and zinc ion are colored salmon and purple, respectively. (b) Ribbon diagram showing the monomer of the ZBD and details of bound XB peptide. Side chains of 5 cysteines in the ZBD and XB peptide are shown.

References

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[2] D.N. Bolon et al., Mol. Cell 13, 443-9 (2004).
* hksong@korea.ac.kr