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Crystal structure of Atg10 from *Saccharomyces cerevisiae*

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1 Introduction

Autophagy is a process for intracellular degradation of the components using lysosomal machinery in eukaryotic cells. The ubiquitin-like (UBL) conjugation by core-autophagic molecules plays a critical role for autophagy. UBLs, Atg8 and Atg12 are activated by the same E1 enzyme, Atg7. Then E1 directs the UBLs to E2 enzymes, Atg3 and Atg10, respectively. Then, the UBLs are conjugated with phosphatidylethanolamine (PE) and Atg5, respectively. The Atg10 is involved in Atg12-Atg5 conjugation reaction, however its structure was not unveiled. We obtained protein crystals of the Atg10 from *Saccharomyces cerevisiae* using a heavy-atom derivatization method and the crystal structure is determined at 2.7 Å resolution. Although sequence similarities among Atg10, Atg3 and other E2 enzymes are about 20% or less, the crystal structure of Atg10 resembles a canonical E2 core domain [1].

2 Methods

Full-length Atg10 was expressed as a GST fusion form in BL21(DE3) cell and purified by glutathione affinity chromatography followed by treatment with TEV protease at a molar ratio of 1:10. Then, cleaved protein products were purified by anion-exchange chromatography and separated from GST protein using glutathione affinity column. Finally, Atg10 was purified using a Superdex75 gel-filtration column pre-equilibrated with 50 mM Tris-HCl pH 8.0, 500 mM NaCl, and 2 mM TCEP. Sample was concentrated to about 10 mg/ml and crystallized using hanging drop vapor diffusion method at 295 K, mixing of an equal volumes of protein and reservoir solution containing 50 mM bis-Tris-HCl pH 6.15, 3.0 M NaCl, and 1 mM 1,4-diacetoxymercury-2,3-dimethoxybutane. Crystals were grown within 3 days and transferred to reservoir solution containing 25%(v/v) glycerol and then kept in liquid nitrogen before data collection at NW12. Initial phases were calculated and an initial model was partially built using PHENIX software package. The model was rebuilt manually using Coot and O. Refinement was also carried out using the PHENIX program.

3 Results

The diffraction of native crystal without any heavy-atom was limited to only 4.5 Å resolution, but mercury-derivatized crystal diffracted to 2.7 Å resolution [1]. A significant improvement of diffraction limits by heavy-atom derivatization was essential for the structure determination. The crystal structure of Atg10 was determined by the MAD method and refined to a R_{work} and R_{free} of 0.223 and 0.251, respectively. Approximate dimensions of Atg10 are 65 Å × 41 Å × 40 Å. It consists of 3 α-helixes and 7 β-strands and has an elongated shape in one direction (Fig. 1a). But 10 residues (from Asn49 to Lys58) on the surface loop and two C-terminal residues (Asp166 and Ser167) could not be built. Overall structure of Atg10 is similar to Atg3 and other E2 ubiquitin conjugating enzymes but amino acid sequences of them are quite different (less than 20% sequence identity). The highest structural similarity using the DALI server is Atg3 (Z = 11.6), another E2-like enzyme in autophagy and ubiquitin E2-enzyme UbcH7 also possesses similar structure (Z = 5.2). The key residue Cys133 is located in a loop region between β7 strand and α3 helix. And the Hg atom is grabbed by 2 cysteine residues, Cys133 and Cys137 (Fig. 1b). So it may stabilize the flexible loop and improves crystal formation. As expected, the key cysteine residue is exposed to the solvent.

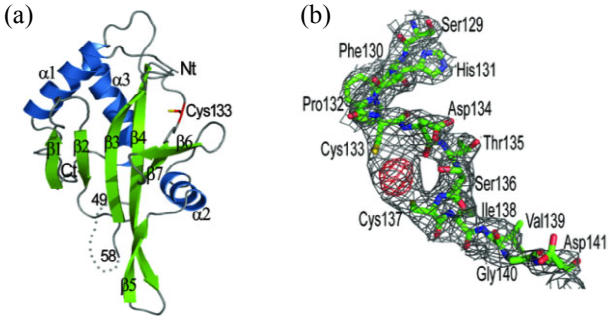

Fig. 1: The crystal structure of Atg10.
(a) Ribbon diagrams showing the secondary-structure elements of Atg10. Seven β-strands (green arrows), three α-helices (blue ribbons) and connecting loops (grey) are shown. The catalytic residue Cys133 is represented in stick form.
(b) Electron-density map near the catalytic residue Cys133 of Atg10. The final 2Fo–Fc map (grey) and the Fo–Fc OMIT map for the bound Hg atom (red) were calculated using 27.8–2.7 Å resolution data and were contoured at 1.2σ and 6.0σ, respectively. The two free cysteine residues Cys133 and Cys137 are covalently modified by a heavy-metal atom.

Reference

[1] S.B. Hong *et al.*, *Acta Crystallogr. D* **68**, 1409 (2012).

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