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Crystal structure of RavZ from *Legionella pneumophila*

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1 Introduction

Hosts utilize macroautophagy/autophagy to clear invading bacteria; however, bacteria have also developed a specific mechanism to survive by manipulating the host cell autophagy mechanism. One pathogen, *Legionella pneumophila*, can hinder host cell autophagy by using the specific effector protein RavZ that cleaves phosphatidylethanolamine-conjugated LC3 on the phagophore membrane. [1] However, the detailed molecular mechanisms associated with the function of RavZ have hitherto remained unclear. Here, we report on the biochemical characteristics of the RavZ-LC3 interaction, the solution structure of the 1:2 complex between RavZ and LC3, and crystal structures of RavZ showing different conformations of the active site loop without LC3. Based on our biochemical, structural, and cell-based analyses of RavZ and LC3, both distant flexible N- and C-terminal regions containing LC3-interacting region (LIR) motifs are important for substrate recognition. These results suggest a novel mechanism of RavZ action on the phagophore membrane and lay the groundwork for understanding how bacterial pathogens can survive autophagy. [2]

2 Experiment

His-tagged RavZ from *Legionella pneumophila* was expressed and GST-tagged LC3 from *Homo sapiens* using modified pGEX4T-1 vector. The overexpressed RavZ protein was purified by nickel-NTA affinity chromatography followed by anion exchange and size exclusion chromatography. And the overexpressed LC3B protein was purified by GST chromatography and removed GST tag by TEV protease. For further purification, we performed cation exchange and size exclusion chromatography. RavZ was crystallized using the hanging drop method by vapor-diffusion at 20 ºC. In RavZ crystal diffracted up to 2.7 Å and the number of molecules in asymmetric unit was one. The space group and unit cell parameters were I422 with a=b=222.0 Å and c=71.3 Å. Diffraction data were collected using an ADSC quantum CCD detector at the NW12 beamline of Photon Factory. 180-200 images were collected with 1º oscillation, and each image was exposed for 1-3 seconds. The diffraction data were processed and scaled using the HKL2000 software package. Phases were obtained by single wavelength anomalous dispersion (SAD) using MOLREP in the CCP4 program suite. The initial model was manually built and refined using COOT and PHENIX.

3 Results

Legionella pneumophilia RavZ consists of two distinct domains which are catalytic domain and membrane targeting domain. Catalytic domain is similar with human sumo protease and membrane targeting domain has special protein folding only found bacteria proteins (Fig. 1A and 1B). Due to the flexibility, N-terminal 48 residues and C-terminal 71 residues were not visible in the electron density map.

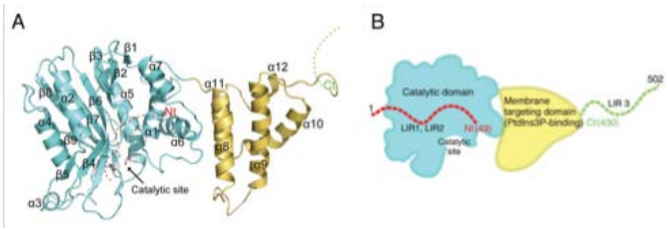

Fig. 1: Crystal structure of RavZ. (A) Overall structures of RavZ. (B) Schematic drawing of RavZ overall structure.

We tried to solve the structure of complex between RavZ and LC3B, but it was not successful in the crystallization. Therefore, we performed the small-angle X-ray scattering experiments using a complex between RavZ and LC3B in solution. According to SAXS model, we found that N-terminus and C-terminus can interact with LC3B separately (Fig. 1C and 1D). The binding stoichiometry between RavZ and LC3B is 1:2.

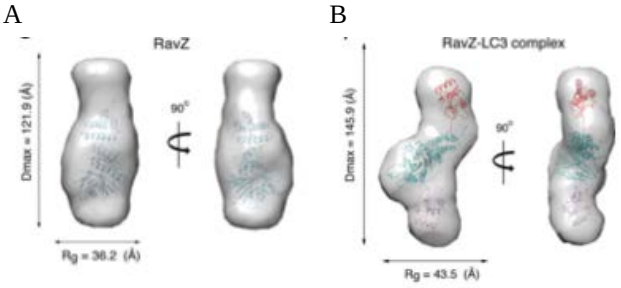

Fig. 2: Solution structure of RavZ-LC3B complex. (A) SAXS model of RavZ. (B) SAXS model of RavZ and LC3B complex.

References

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