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Structural study of the Atg101-Atg13 complex essential for autophagy initiation

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1 Introduction

Autophagy is an intracellular degradation system conserved among most eukaryotes. Upon autophagy induction, such as starvation, dozens of autophagy-related (Atg) proteins are localized to a site of autophagosome formation, among which the Atg1 complex in yeast and ULK complex in mammals function as the most upstream factor and mediate recruitment of downstream factors to the site. Atg101 is a conserved component of the mammalian ULK complex and fission yeast Atg1 complex, but is absent form budding yeast Atg1 complex. The only known function of Atg101 was to bind and stabilize Atg13, another component of the Atg1/ULK complex. In order to reveal the structure and the molecular roles of Atg101 in autophagy, we performed structural studies on the Atg101-Atg13 complex using *Schizosaccharomyces pombe* (Sp) homologs.

2 Experiment

Atg101 and the HORMA domain of Atg13 from fission yeast were expressed in *Escherichia coli* separately, and each protein was purified through several combinations of chromatography. For preparation of the complex, purified Atg101 and Atg13^{HORMA} were mixed and applied to a size-exclusion chromatography.

Small angle X-ray scattering (SAXS) experiments were performed for Atg101 alone, Atg13^{HORMA} alone, as well as the Atg101-Atg13^{HORMA} complex. SAXS data were collected on Photon Factory BL-10C at 293 K using PILATUS 2M detector (DECTRIS) with the sample-to-detector distance of 1.0 m. The wavelength of the X-rays was set to 1.488 Å. Three types of samples with different concentrations were studied by SAXS: 1) 2.6, 5.4, 7.7, 10.4, and 13.1 mg/mL of SpAtg101, 2) 1.1, 2.1, and 3.6 mg/mL of SpAtg13^{HORMA}, and 3) 1.1, 2.6, 3.4, and 4.9 mg/mL of SpAtg101–SpAtg13^{HORMA}. Ovalbumin (Sigma, molecular weight 44 kDa) at the concentration of 1.0, 3.1, and 5.4 mg/mL was used as a standard sample to determine the molecular mass of the proteins. All the samples were measured in buffer consisting of 20 mM HEPES pH 7.5 and 150 mM NaCl. The molecular mass was estimated by comparison of the extrapolated value of the intensity at the origin value, I(0), of the scattering data from the samples with that from Ovalbumin.

3 Results and Discussion

We previously determined the crystal structure of the SpAtg101-SpAtg13^{HORMA} complex, which revealed that Atg101 is also a HORMA protein. It has been established that a representative HORMA protein, Mad2, has two distinct open and closed conformations named O-Mad2 and C-Mad2 and that O-Mad2 and C-Mad2 interact with each other to form a dimer. Crystal structure showed that Atg101 and Atg13 are structurally similar to O-Mad2 and C-Mad2, respectively. However, it remained elusive whether these proteins form homodimers in a similar manner with Mad2. From scattering data collected at different concentrations (Figures 1, 2), we estimated the molecular weight of Atg101, Atg13^{HORMA} and the Atg101-Atg13^{HORMA} complex in solution as 22.2, 21.0 and 41.7 kDa, respectively. These data indicate that Atg101 and Atg13^{HORMA} behave as a monomer, and form a 1:1 heterodimer in solution, and suggest that in contrast to Mad2 that interconverts between open and closed conformations and form the O-Mad2-C-Mad2 homodimer, Atg101 and Atg13 are locked to the open and closed conformations, respectively, and can only form the Atg101-Atg13 heterodimer [1].

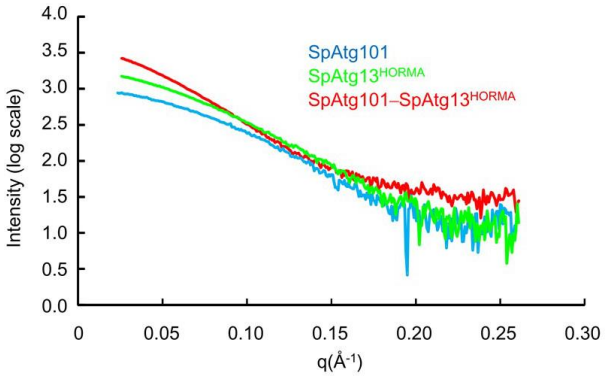

Fig. 1: Experimental scattering patterns of Atg101 alone, Atg13^{HORMA} alone, and the Atg101-Atg13^{HORMA} complex..

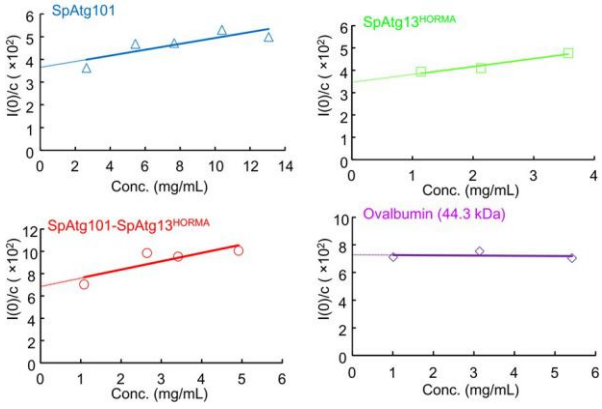

Fig. 2: The concentration dependence of I(0)/Concentration, where I(0) is the forward scattering intensity derived using the Guinier law.

References

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