Biological Science

6A, NW12/2002G149

Crystal structure of GGA3 GAT / ubiquitin complex

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Introduction

GGA (Golgi-localizing, γ-adaptin ear domain homology, ARF-binding) proteins are composed of four functional regions: an N-terminal VHS (Vps27/Hrs/Stam) domain, a GAT (GGA and Tom1) domain, a hinge region and a C-terminal GAE (γ-adaptin ear homology) domain [1]. Recently the GAT domain of GGAs was found to bind ubiquitin. This finding uncovered a novel role for GGAs in ubiquitin-dependent sorting of cargo proteins in the endocytic pathway, in addition to the previously established function of GGAs in sorting lysosomal cargo receptors from the *trans*-Golgi network (TGN) to endosomes. The GAT domain of GGAs consists of two subdomains: N-GAT and C-GAT. N-GAT is a helix-loop-helix structure that is responsible for ADP-ribosylation factor (ARF) binding, whereas C-GAT is a three helix bundle that is responsible for ubiquitin binding. To elucidate the ubiquitin recognition mechanism of C-GAT, we solved the crystal structure of the complex between GGA3 C-GAT and ubiquitin [2].

Materials and Methods

The mixture of equimolar (1 mM each protein) amounts of SeMet-substituted GGA3 C-GAT domain and native bovine ubiquitin was crystallized using the hanging-drop vapour diffusion method. Crystals were obtained using 20% (w/v) PEG3350, 0.3 M ammonium formate and 50 mM MES pH 6.5 as a reservoir solution at 20 °C, and belong to the orthorhombic space group $P2_12_12_1$.

Crystals were cryoprotected in the reservoir solution supplemented with 15% ethylene glycol and frozen in liquid nitrogen. A three-wavelength data set for MAD phasing was collected to 2.9 Å resolution at PF beamline BL-6A. A data set at single wavelength with another crystal was collected to a higher resolution of 2.6 Å at PF-AR NW12 beamline.

The crystal contains four GGA3 C-GAT/ubiquitin complexes in an asymmetric unit. The final model of the complex has an *R*-factor of 22.1 % and an R_{free} of 29.0 %.

Results and discussion

The asymmetric unit of the complex crystal contained four copies of GGA3 C-GAT/ubiquitin heterodimers. The GGA3 C-GAT domain consists of a three-helix bundle (α1, α2 and α3). C-GAT molecules dimerize using helices α2 and α3 in the crystal lattice (Figure 1). Ubiquitin is bound on a hydrophobic patch of helices α1 and α2 of C-GAT. This ubiquitin-binding site of GGA3 C-GAT includes Leu227 and Met231 of helix α1 and Leu247 of helix α2 of GGA3 C-GAT. It interacts with the hydrophobic patch of the ubiquitin Ile44 surface, which is composed of residues of β1, β3, β4 and β5 strands of ubiquitin.

The Ile44 surface of ubiquitin is commonly used as a binding core by a variety of differently folded ubiquitin-binding modules. The hydrophobic patch of the Ile44 ubiquitin surface in the GGA3 C-GAT/ubiquitin complex can be divided into three hydrophobic pockets I, II and III. Pocket I is a large cavity formed by the side chains of ubiquitin Leu8, Ile44, His68 and Val70. Pocket II is a shallow concave like the seat of a saddle, formed by the side chains of Ile44 and Gln49, and the main chains of Gly47 and Lys48. Pocket III is another large cavity formed by the side chains of Arg42, Ile44, Gln49 and Val70. Pockets I, II and III accommodate the side chains of the hydrophobic residues Leu227, Met231 and Leu247 of GGA3 C-GAT, respectively. We compared the known structures of the Ile44 surface of ubiquitin in complex with various ubiquitin-binding modules and found that the three hydrophobic pockets (I, II and III) of the Ile44 surface accommodate generally hydrophobic residues in all cases.

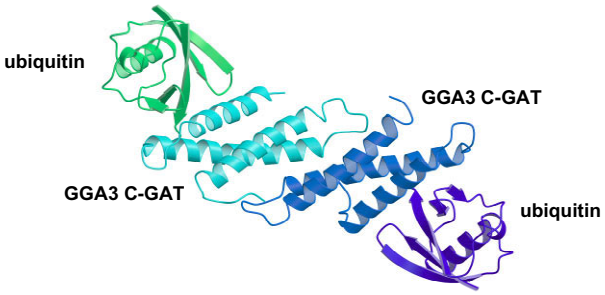

Fig. 1 GGA3 C-GAT molecules dimerize in the crystal lattice of the GGA3 C-GAT/ubiquitin complex.

References

[1] K. Nakayama and S. Wakatsuki, Cell Struct. Funct. 28, 431 (2003).
[2] M. Kawasaki et al., Genes Cells, in press
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