Crystallography

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Crystal structure of the Nas6-Rpt3 complex of the yeast 26S proteasome

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Introduction

Appropriate control of protein degradation is critical for the maintenance and regulation of cellular events [1,2]. Many cellular proteins are degraded by the 26S proteasome, an evolutionarily-conserved cylindrical organelle, after they have been covalently attached to polyubiquitin. The 26S proteasome can be divided into the 20S core and 19S regulatory particles. The 20S core particle has a barrel-like structure comprising four rings, each with seven subunits. The 19S regulatory particle has a base comprising six proteasomal ATPases (Rpt1 to Rpt6 in yeast), two additional non-ATPase subunits (Rpn1 and Rpn2), and a lid structure composed of at least 8 non-ATPase subunits, which is assumed to be connected to the base by the Rpn10 subunit. Importantly, the subunit composition of the 26S proteasome is highly conserved from yeast to human. The tertiary structures of the 20S core particles from different species are available; however, that of the 19S regulatory particle has not yet been solved.

Recently, the S6 ATPase (orthologue of yeast Rpt3), which is one of the ATPases of the human 19S regulatory particle, was shown to interact with the oncoprotein gankyrin (Nakamura et al., 2007). Recent studies suggest that gankyrin regulates the degradation of pRb and p53, and possibly stimulates the recruitment of these tumor suppressors to the 26S proteasome for subsequent degradation.

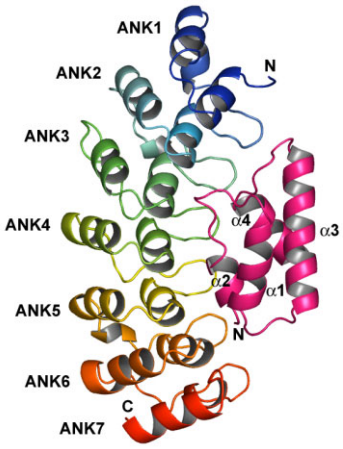

Figure 1. Tertiary structure of the Nas6 – Rpt3-C complex. Ribbon diagram of the Nas6 – Rpt3-C complex. Each ankyrin repeat of the gankyrin is indicated by ANK1 – 7 (blue to red). The Rpt3-C tertiary structure is in pink. The N and C termini of both molecules are indicated.

The yeast orthologue of gankyrin, encoded by YGR232w, was initially purified as the non-ATPase subunit 6 (Nas6) involved in the 19S regulatory complex. Though the function of gankyrin has been well characterized, the role of its yeast orthologue Nas6 is still elusive. In this study, we report the crystal structure of the complex of Nas6 and a C-terminal domain of the proteasomal ATPase Rpt3 (Rpt3-C, hereafter), which is essential for the interaction with Nas6.

Materials and Methods

The cloning, protein expression, purification and crystallization of the Nas6 – Rpt3-C complex was described elsewhere [3]. X-ray diffraction data sets, collected at the beamline NW12 with a Quantum 210 detector (KEK, Tsukuba, Japan), were integrated and scaled with HKL2000. The structure of the Nas6 – Rpt3-C complex was solved by the molecular replacement method, using the apo-form of the Nas6 structure as a search model. The atomic coordinate has been deposited in the Protein Data Bank with the accession code 2DZN.

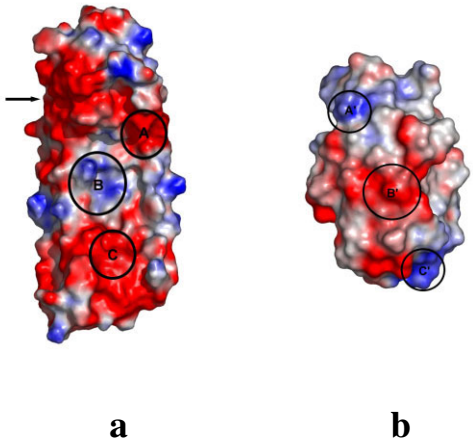

Figure 2. The interface between Nas6 and Rpt3. (**a** and **b**) Charge distribution on the surfaces of Nas6 (**a**) and Rpt3-C (**b**). The complementary surface patches responsible for complex formation are indicated by circles (labeled as A, B and C in Nas6, and as A', B' and C' in Rpt3-C, respectively). Red, blue and white represent acidic, basic, and neutral, respectively. An arrow indicates a proposed potential site for another Nas6 partner.

Results and Discussion

The Nas6 protein forms an elongated shaped structure with dimension of 74 x 36 x 33 Å, consisting of seven ankyrin repeats, ANK1 - 7 (Fig. 1). Each ankyrin repeat, which adopts the tandem ankyrin repeat fold, is formed by approximately 30 amino acid residues with two antiparallel α-helices followed by a β-hairpin, except for ANK1, which lacks the β1 strand. The adjacent repeats stack together and resemble a cupped hand, with the β-hairpins corresponding to the fingers and the helices as the palm. The α1 helices of the ankyrin repeats face inward and reside on the concave side, while the α2 helices of the ankyrin repeats lie on the convex side of the elongated structure.

The C-terminal domain of Rpt3 mainly consists of α-helices (α1, α2, α3 and α4) that form an α-helical bundle (Fig. 1). The α-helical bundle is stabilized through intramolecular hydrophobic forces. The α1 helix and the larger α3 helix are nearly parallel to each other. The shorter α2 and α4 helices are placed over the α1 and α3 helices, respectively. The α1 and α2 helices are connected by a long loop comprising about 10 amino acid residues.

The Rpt3-C molecule binds into the concave region of Nas6 (Fig. 1). The α1 helix and β-hairpin loop of the first six ankyrin repeats, ANK1-6, of Nas6 make contact with α2 and α4 helices, and the loops α1/α2, α2/α3 and α3/α4 of Rpt3-C. The N-terminal end of the α1-helix also forms substantial interactions with the Nas6 protein. Intriguingly, the concave region of Nas6 is almost completely occupied by the Rpt3-C molecule. The interacting surfaces of these two proteins are highly complementary to each other in terms of the charged residues. An electrostatic surface analysis revealed that the interacting region of Nas6 contains two negatively- and one positively charged patches (Fig. 2a), while that of Rpt3-C contains two positively- and one negatively charged patches (Fig. 2b).

We have recently determined the crystal structure of gankyrin in complex with the C-terminal domain of S6 [5]. As expected, the overall structures of these two protein complexes, as well as the mode of interactions with their counterparts, are similar. The charged patches on the interface and the number of salt links are nearly conserved between these complexes. Structural and biochemical evidences presented in this study confirmed that Nas6 was indeed a subunit of the 26S proteasome, through its association with the Rpt3 subunit of the ATPase ring of the 19S regulatory particle. The structural basis will aid in identification and characterization of possible substrates for 26S proteasome-dependent protein degradation in yeast.

References

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