Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Solution structure of the N-terminal domain of proteasome lid subunit Rpn5

Wenbo Zhang [a, c], Cong Zhao [b, c], Yunfei Hu [b, c, **], Changwen Jin [a, b, c, d, *]

[a] College of Life Sciences, Peking University, Beijing 100871, China
[b] College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
[c] Beijing Nuclear Magnetic Resonance Center, Peking University, Beijing 100871, China
[d] Beijing National Laboratory for Molecular Sciences, Peking University, Beijing 100871, China

ARTICLE INFO

Article history:
Received 14 August 2018
Accepted 26 August 2018
Available online xxx

Keywords:
Proteasome
Regulatory particle
Rpn5
NMR
Solution structure

ABSTRACT

The 26S proteasome is the major protein degradation machinery in living cells. The Rpn5 protein is one scaffolding subunit in the lid subcomplex of the 19S regulatory particle in the proteasome holoenzyme. Herein we report the solution structure of the N-terminal domain (NTD) of yeast Rpn5 at high resolution by NMR spectroscopy. The results show that Rpn5 NTD adopts α-solenoid-like fold in right-handed superhelical configuration formed by a number of α-helices. Structural comparisons with currently available cryo-EM structures reveal local structural differences in the first three helices between yeast and human Rpn5. The results further highlight the conformational flexibility in three possible protein interaction sites. Moreover, the structures of the NTD show large variations among different PCI-containing Rpn subunits. Our current results provide atomic-level structural basis for further investigations of protein-protein interactions and the proteasome assembly pathway.

1. Introduction

The 26S proteasome is an ATP-dependent multi-subunit protease responsible for degrading polyubiquitin-tagged protein in eukaryotic cells, and plays essential roles in various biological processes [1–3]. This 2.5 MDa multi-protein complex comprises a 20S core particle (CP) that contains the cylindrical chamber for protein degradation, and two 19S regulatory particles (RP) that cap the 20S CP on both ends and function in substrate recruitment, deubiquitination, unfolding and translocation into the CP chamber [4]. The RP is a 19-subunit protein complex and can be further divided into the “base” and “lid” subcomplexes. The base subcomplex directly associates with the CP, whereas the lid locates at the distal end. The lid subcomplex consists of 9 non-ATPase subunits (Rpn3, 5–9, 11–12 and Rpn15/Sem1/DSS1) [5], among which Rpn11 shows the deubiquitylation (DUB) activity [6].

Based on a series of cryo-EM investigations on the 26S proteasome in recent years, the molecular architecture of this central proteolytic machinery, including the lid subcomplex in either the free state or in the context of the protease holoenzyme, has been elucidated to near-atomic resolution [7–15]. The structures reveal a palm-like (or horseshoe-shaped) scaffold in the lid subcomplex formed by the Rpn3, Rpn5, Rpn6, Rpn7, Rpn9 and Rpn12 subunits. These subunits share a homologous C-terminal domain, which is also present in the COP9/signalosome and the initiation factor eIF3, and thus termed the Proteasome-CSN-eIF3 (PCI) domain. The Rpn8 and Rpn11 subunits, both of which harbor an Mpr1-Pad1 N-terminal (MPN)-domain, form a heterodimer and sits in the center of the palm, whereas the N-terminal α-solenoid domains of all PCI-containing subunits extend out radially like six fingers.

Among the six PCI domain-containing subunits, Rpn5 was found to be essential for the integrity and efficiency of the 26S proteasome [16–18]. The cryo-EM structures of proteasome holoenzyme show that Rpn5 interacts with Rpn9 and Rpn6 via its PCI domain, and it also contacts subunits in the base of the RP and the CP via the N-terminal domain (NTD) [8,10,15,19]. On the other hand, the conformation of Rpn5 is significantly different in the context of the 26S proteasome holoenzyme compared to in the isolated lid subcomplex [8,15]. The Rpn5 finger is curled up in free lid subcomplex

* Corresponding author. Beijing Nuclear Magnetic Resonance Center, College of Life Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.
** Corresponding author. Beijing Nuclear Magnetic Resonance Center, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.
E-mail addresses: yunfei@pku.edu.cn (Y. Hu), changwen@pku.edu.cn (C. Jin).

https://doi.org/10.1016/j.bbrc.2018.08.159
0006-291X/

and it occludes the active site of Rpn11 prior to incorporation into the proteasome. Upon forming the proteasome holoenzyme, the Rpn5 finger swings down to contact the 20S CP and adopts an extended conformation. Furthermore, the Rpn5 NTD undergoes substantial movements in isolated lid complex demonstrated by cross-linking experiments [20]. In particular, Lys108 in the Rpn5 NTD cross-linked with residues in the Rpn3, Rpn6, Rpn8 and Rpn11 subunits in the isolated lid, the distances of which are much farther apart in the proteasome holoenzyme. The extremely high flexibility of the Rpn5 NTD suggests that it may play important roles in regulating the proteasome assembly.

Although near-atomic resolution (3–5 Å) could be obtained for the 20S CP and part of the 19S RP in the cryo-EM structures of 26S proteasomes, resolutions of the PCI domain-containing subunits are significantly lower, and the N-terminal regions are particularly not well-resolved [13–15,19,21,22]. To gain deeper insights into the proteasome assembly as well as the functions of the lid subunits, more detailed structural information is needed. Herein, we determined the structure of the NTD (residues 1–136) of *Saccharomyces cerevisiae* Rpn5 using solution NMR method. Our results provide atomic-resolution details of this highly flexible domain, and highlights three structural regions that may play important roles in mediating protein-protein interactions.

2. Materials and methods

2.1. Protein expression and sample preparation

The *S. cerevisiae rpn5* gene was cloned into a pET28a expression vector with a C-terminal His_6-tag and expressed in *Escherichia coli* BL21 (DE3) strain (Novagen). *E. coli* cells were grown in 1 L Luria-Bertani (LB) with kanamycin (50 mg/L) at 35 °C until the OD_{600} reached ~0.8. After centrifugation at 4000 g at 4 °C, the cells were re-suspended in 500 ml M9 medium, and isopropyl-β-D-thio-galactoside (IPTG) was added to a final concentration of 0.4 mM to induce protein expression. The cells were grown for another 10 h at 25 °C before harvesting. For preparations of $^{13}C/^{15}N$-labeled Rpn5 proteins, $^{15}NH_4Cl$ and $^{13}C_6$-glucose were used in the M9 minimal medium.

The Rpn5 protein was purified by Ni-NTA affinity chromatography followed by gel filtration (Superdex-200) using the ÄKTA FPLC system (GE Healthcare Life Sciences). The protein was then digested by trypsin (with Rpn5: trypsin mass ration 200:1) at 4 °C in a buffer containing 50 mM sodium phosphate (pH 7.0) and 100 mM NaCl and was further purified using a Superdex-75 gel filtration column. A stable fragment with a molecular weight of ~16 kDa was identified to correspond to the 1–136 fragment of Rpn5 ($Rpn5_{1\text{-}136}$), and was further conformed by NMR analysis of a construct directly expressing this segment. NMR samples were prepared with uniformly ^{15}N-/^{13}C-labaled $Rpn5_{1\text{-}136}$ in a buffer containing 50 mM sodium phosphate buffer (pH 6.0), 50 mM Na_2SO_4, 2% (v/v) trifluoroethanol, 5% (w/v) sucrose and 1 mM dithiothreitol (DTT). D_2O was added to 5% for field lock and 0.01% 2,2-dimethyl-2-silapen-tanesulfonic acid (DSS) was used as the internal chemical shift reference.

2.2. NMR spectroscopy

All NMR experiments were performed at 25 °C on Bruker Avance 500, 800 and 950 MHz spectrometers equipped with cyroprobes. Conventional triple-resonance NMR spectra were acquired to obtain chemical shift assignments [23]. Distance restrains were derived from 3D ^{15}N- and ^{13}C-edited NOESY-HSQC spectra collected with 100 ms mixing time. All spectra were processed with NMRPipe [24] and analyzed with NMRView [25]. The backbone steady-state heteronuclear $\{^1H\}$-^{15}N NOE values of $Rpn5_{1\text{-}136}$ were measured on a Brucker Avance 800-MHz NMR spectrometer at 25 °C [26].

2.3. Structure calculation

The structures of $Rpn5_{1\text{-}136}$ were calculated using the program CYANA [27] and refined with AMBER [28]. Distance restraints were derived from inter-proton nuclear Overhauser effect (NOE). Dihedral angles (φ and ψ) were predicted based on the chemical shifts using program TALOS [29]. The initial structures were generated using the CANDID module of CYANA [30], and 20 structures with the lowest energies were selected as models for the program SANE to extend the NOE assignments [31]. 200 structures were first calculated by CYANA, and the 100 lowest energy structures were further refined by AMBER. Finally, the 20 lowest-energy conformers were selected as the representative structures. The quality of the structures was verified using the PROCHECK_NMR software [32].

3. Results and discussion

3.1. Solution structure of yeast $Rpn5_{1\text{-}136}$

The *S. cerevisiae* Rpn5 is a 445-residue protein with a domain structure comprising an N-terminal all-helical region, a PCI domain and a C-terminal helical tail (Fig. 1A). Limited proteolysis by trypsin reveals a relatively stable fragment corresponding to residues 1–136, which we herein term as the NTD of Rpn5 or $Rpn5_{1\text{-}136}$. The solution structure of $Rpn5_{1\text{-}136}$ was calculated using a total of 3801 NOE-derived distance restraints and 224 dihedral angle restraints. A superimposition of the 20 lowest-energy conformers and the ribbon diagram of the lowest-energy structure are shown in Fig. 1B and C, respectively. The structural statistics is summarized in Table 1. Atomic coordinates of the structures have been deposited in the Protein Data Bank (PDB) under the accession code 5ZMR.

$Rpn5_{1\text{-}136}$ adopts an all-helical folding comprising of six anti-parallel α-helices (α1: Ser14-Asn30; α2: Ala32-Ala49; α3: Ala53-Ser68; α4: Trp72-Lys84; α5: Ser92-Leu104 and α6: Leu112-Glu127), forming a continuous right-handed superhelical α-solenoid similar to the tetratricopeptide repeats (TPR) [33], whereas the N- and C-terminal regions (Met1-Tyr13 and Asn128-Arg136) are unstructured. Among the loops connecting the α-helices, the loops connecting α4-α5 (85–91) and α5-α6 (105–111) are relatively long, both comprising 7 residues. The helices are packed together via a network of hydrophobic interactions mainly involving leucine, isoleucine and valine residues (Fig. 1D).

3.2. Conformational flexibility

$Rpn5_{1\text{-}136}$ adopts an overall rigid conformation, with a backbone root mean square deviation (RMSD) of 0.6 ± 0.2 Å for residues in regular secondary structures. The structural rigidity is also reflected by the backbone heteronuclear $\{^1H\}$-^{15}N NOE values, with residues in regular secondary structures showing $\{^1H\}$-^{15}N NOE values > 0.75 (Fig. 1E). Apart from the N- and C-termini, the α4-α5 and α5-α6 loops show relatively high internal flexibility, with larger than the average RMSD and reduced heteronuclear $\{^1H\}$-^{15}N NOE values. Moreover, a number of residues such as Cys32, His86-Lys90 and Lys108 show broadened NMR resonances, indicative of intermediate conformational exchanges on the NMR timescale.

3.3. Comparison with the cryo-EM structures

In the cryo-EM studies on both yeast and human proteasomes

Please cite this article in press as: W. Zhang, et al., Solution structure of the N-terminal domain of proteasome lid subunit Rpn5, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.08.159