1.15 Å resolution structure of the proteasome-assembly chaperone Nas2 PDZ domain

Chingakham R. Singh, Scott Lovell, Nurjahan Mehzabeen, Wasimul Q. Chowdhury, Eric S. Geanes, Kevin P. Battaile and Jeroen Roelofs


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The 26S proteasome is a 2.5 MDa protease dedicated to the degradation of ubiquitinated proteins in eukaryotes. The assembly of this complex containing 66 polypeptides is assisted by at least nine proteasome-specific chaperones. One of these, Nas2, binds to the proteasomal AAA-ATPase subunit Rpt5. The PDZ domain of Nas2 binds to the C-terminal tail of Rpt5; however, it does not require the C-terminus of Rpt5 for binding. Here, the 1.15 Å resolution structure of the PDZ domain of Nas2 is reported. This structure will provide a basis for further insights regarding the structure and function of Nas2 in proteasome assembly.

1. Introduction

The eukaryotic proteasome consists of a core particle (CP) and a regulatory particle (RP). The CP is a cylindrically shaped structure with the proteolytic active sites on the inner surface of this complex (Groll et al., 1997). One or both ends can associate with the RP, forming the 26S proteasome. CP assembly and RP assembly are both assisted by proteasome-specific chaperones (Bedford et al., 2010). The RP-specific chaperones Nas6, Nas2, Hsm3 and Rpn14 are important for the assembly of a hetero-hexameric AAA-ATPase ring formed by the subunits Rpt1 to Rpt6 (Funakoshi et al., 2009; Kaneko et al., 2009; Le Tallec et al., 2009; Roelofs et al., 2009; Saeki et al., 2009). Interestingly, each chaperone largely consists of protein–protein interaction motifs: Nas6 contains seven ankyrin repeats, Hsm3 is formed by armadillo/heat-like repeats, Rpn14 contains a WD40 domain and Nas2 has a predicted PDZ domain. Although structurally different, each chaperone binds to the C-domain of a specific Rpt protein. Structural studies looking at the interaction between Nas6 and Rpt3 (Nakamura et al., 2007; Roelofs et al., 2009), Hsm3 and Rpt1 (Barrault et al., 2012; Takagi et al., 2012; Park et al., 2013), and Rpn14 and Rpt6 (Ehlinger et al., 2013) have provided substantial structural insights. However, no structural data for Nas2, or the human ortholog p27/PSMD9, have been published to date. Binding studies between Nas2 and Rpt5 have shown that deletion of the C-terminal residue of Rpt5 did not impact Nas2 binding (Lee et al., 2011). Deletion of the last three amino acids, on the other hand, caused a strong reduction in binding. This indicates that the tail of Rpt5 is important for binding with Nas2 (Lee et al., 2011). This same tail is also involved in the association between the Rpt5 subunit and the CP (Tian et al., 2011; Beck et al., 2012; Lander et al., 2012), which suggests that the binding of Nas2 prevents the association of Rpt5, or Rpt5-containing complexes, with the CP. The structure of the Nas2 PDZ domain reported here is the first step towards a structural understanding of this interaction.

2. Materials and methods

2.1. Macromolecule production

The open reading frame of *Saccharomyces cerevisiae* Nas2 was subcloned into a pGEX-6P1-derived plasmid, creating plasmid pJR500 (Lee et al., 2011). Based on domain and secondary-structure prediction analyses, two truncated versions of Nas2 containing the single PDZ domain were generated: Nas2LND, covering amino acids 1.15 Å resolution structure of the proteasome-assembly chaperone Nas2 PDZ domain

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2. Materials and methods

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Gln126-Leu220 (pJR607), and Nas2ND, covering Asn91–Leu220 (pJR606). Expression and purification of the different proteins were conducted as described previously for Hsm3 (Park et al., 2013). Basically, Escherichia coli cells induced to express the GST-fused proteins were lysed. The fusion proteins were bound to glutathione resin. The affinity tag was removed by incubation with PreScission protease and the eluted proteins were further purified by size-exclusion chromatography using a Superdex 200 (10/300 GL) column.

2.2. Crystallization

A purified sample of Nas2LND (Table 1) concentrated to 7.0 mg ml\(^{-1}\) in 50 mM NaCl, 50 mM Tris–HCl pH 6.8, 1 mM DTT, 1 mM EDTA was used for crystallization screening. All crystallization experiments were conducted by sitting-drop vapor diffusion in Compact Jr plates (Emerald Bio) using equal volumes of protein and crystallization solution and 25%\(v/v\) sodium acetate/acetic acid pH 7.0 mg ml\(^{-1}\). Crystal samples were transferred to a fresh drop composed of 75% PEG 2000 MME, 100 mM sodium acetate/acetic acid pH 4.0 mm in length were obtained at 4°C from Wizard Classic 4 screen (Emerald Bio) condition No. 26 [10%\(w/v\) PEG 2000 MME, 100 mM sodium acetate/acetic acid pH 5.5, 200 mM ammonium sulfate].

2.3. Data collection and processing

Crystal samples were transferred to a fresh drop composed of 75% crystallization solution and 25%\(v/v\) PEG 400 and stored in liquid nitrogen. Diffraction data were collected in-house using a Rigaku RU-H3R rotating-anode generator (Cu Ka) equipped with Osmic Blue confocal mirrors and an R-AXIS IV++ image-plate detector.

High-resolution data were collected on beamline 17-ID at the Advanced Photon Source using a Dectris Pilatus 6M pixel-array detector. Intensities were integrated using XDS (Kabsch, 2010a,b) and Laue class analysis and data scaling was performed with AIMLESS (Evans, 2011; Evans & Murshudov, 2013), which indicated that the highest probability Laue class was 4\(mnm\) and the most likely space groups were \(P4_2_2_2\) or \(P4_2_2_2\). Data-collection statistics are given in Table 2.

2.4. Structure solution and refinement

The Matthews coefficient (Matthews, 1968) indicated that the asymmetric unit contained a single molecule (\(V_{	ext{mt}} = 1.99\ A^3\ Da^{-1}\), ~40% solvent). Structure solution was conducted by molecular replacement with BALBES (Long et al., 2008), in which the top solution was obtained using the GRASP55 GRASP domain (PDB entry 3rle; Trusche1 et al., 2011) as the search model. Following initial refinement with PHENIX (Adams et al., 2010) the \(R\) and \(R\text{free}\) converged at 42.8 and 46.4%, respectively. The model was improved

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Table 1

Amino-acid sequence of the Nas2LND fragment that crystallized.

<table>
<thead>
<tr>
<th>Source organism</th>
<th>S. cerevisiae strain ATCC 204508/S288c</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA source</td>
<td>S. cerevisiae strain ATCC 204508/S288c</td>
</tr>
<tr>
<td>Expression vector</td>
<td>pGEX-6P1-derived plasmid</td>
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<tr>
<td>Expression host</td>
<td>E. coli BL21(DE3)</td>
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<tr>
<td>Complete amino-acid sequence of the construct produced</td>
<td>GPLETRRASVGS</td>
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Table 2

Data-collection statistics for Nas2LND refined to 1.15 Å resolution.

<table>
<thead>
<tr>
<th>Unit-cell parameters (Å, (^{\circ}))</th>
<th>(a = 39.97, b = 39.97, c = 115.80), (\alpha = \beta = \gamma = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>(P4_2_2_2)</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>39.97–1.15 (1.17–1.15)</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>−173</td>
</tr>
<tr>
<td>Observed reflections</td>
<td>808924</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>33545</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>23.0 (3.4)</td>
</tr>
<tr>
<td>Multiplicity</td>
<td>97.9 (95.1)</td>
</tr>
<tr>
<td>(R\text{merge}^\dagger) (%)</td>
<td>7.7 (107.0)</td>
</tr>
<tr>
<td>(R\text{merge}^\ddagger) (%)</td>
<td>7.8 (110.0)</td>
</tr>
<tr>
<td>(PCC_1)</td>
<td>1.6 (25.6)</td>
</tr>
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</table>

Table 3

Structure refinement.

<table>
<thead>
<tr>
<th>Reflections (working/test)</th>
<th>31796/1698</th>
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<tbody>
<tr>
<td>Resolution (Å)</td>
<td>32.90–1.15</td>
</tr>
<tr>
<td>(R\text{factor} / R\text{free}^\dagger) (%)</td>
<td>17.2/19.4</td>
</tr>
<tr>
<td>(PCC_{1/2}^\ddagger) (%)</td>
<td>0.999 (0.880)</td>
</tr>
</tbody>
</table>

---

Figure 1

Structure of the Nas2 PDZ domain. Asymmetric unit of Nas2LND colored by secondary structure: sheet, magenta; helix, cyan. The disordered region is indicated by the dashed line. N-terminal residues resulting from cloning are colored blue.
3. Results and discussion

3.1. Structure of the Nas2 PDZ domain

The C-terminal half of Nas2 constitutes a PDZ domain, while the N-terminal half has no predicted domain structure (Supplementary Fig. S1a). Nas2 directly interacts with the proteasomal ATPase subunit Rpt5, requiring the last three amino acids of Rpt5 for binding (Lee et al., 2011). Since many PDZ domains bind to C-termini (Lee & Zheng, 2010), this suggests that the Nas2 PDZ domain is responsible for the interaction with Rpt5. Surprisingly, however, deletion of only the ultimate C-terminal residue of Rpt5 had little impact on Rpt5–Nas2 binding (Lee et al., 2011). To gain a structural understanding of the interaction between Nas2 and Rpt5, we attempted to crystallize or co-crystallize Nas2 with the C-domain of Rpt5. Since no crystals were obtained, we created two truncated forms of Nas2 with the goal of minimizing the sequence around the PDZ domain. The truncated forms of Nas2 retained their ability to bind to the Rpt5 C-domain and showed only a modest reduction in affinity as measured using bio-layer interferometry (Supplementary Fig. S1b). Nas2LND produced well diffracting crystals after two months, enabling us to determine a structure comprised of amino acids Gln129–Leu220 in the asymmetric unit (Fig. 1). Nas2LND adopts a fold consisting of five β-sheets and two α-helices commonly found amongst PDZ-domain structures (Fig. 1). N-terminal residues from cloning that spanned Gly115–Gly124 could be traced in the electron-density maps (blue in Fig. 1).

Figure 2
Mapping residues that are conserved between Nas2 and the human ortholog PSMD9. (a) Alignment of Nas2 PDZ-domain residues with the human ortholog PSMD9. Residues modeled in the crystal structure of Nas2 were used in a BLASTp search against the human protein RefSeq database. The top hit was the human ortholog of Nas2, PSMD9, showing 42% identity and 64% conserved residues (the latter are indicated with +). (b) Graphic structure of Nas2 in green. Based on an alignment between Nas2 and PSMD9, conserved residues were colored orange and identical residues red. The cloning-derived residues are in blue. (c) Surface representation of (b). Note the conservation in the groove of the PDZ domain between α-helix 2 and β-strand 5. In PDZ-domain proteins this region commonly binds peptides/C-termini from their binding partner.

Supporting information has been deposited in the IUCr electronic archive (Reference: HV5251).

Table 2

<table>
<thead>
<tr>
<th>Structure</th>
<th>α1</th>
<th>β1</th>
<th>α2</th>
<th>β2</th>
<th>α3</th>
<th>β3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nas2LND</td>
<td>134 FAFIEVPGPSDKADIKVDDSLIGNVHANSHKLSQNTQMVKNEDRPLPVIL</td>
<td>193 FA ++ + PGGP+ A ++VDD+++ G+V+ N L NI VV +E +PL V +R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSMD9</td>
<td>136 FAKVNSISPSPASIALGQDEIVEFGYNQFQQLHNLSVQWSEGKLN</td>
<td>195</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. S1a) Nas2 directly interacts with the proteasomal ATPase subunit Rpt5, requiring the last three amino acids of Rpt5 for binding (Lee et al., 2011). Since many PDZ domains bind to C-termini (Lee & Zheng, 2010), this suggests that the Nas2 PDZ domain is responsible for the interaction with Rpt5. Surprisingly, however, deletion of only the ultimate C-terminal residue of Rpt5 had little impact on Rpt5–Nas2 binding (Lee et al., 2011). To gain a structural understanding of the interaction between Nas2 and Rpt5, we attempted to crystallize or co-crystallize Nas2 with the C-domain of Rpt5. Since no crystals were obtained, we created two truncated forms of Nas2 with the goal of minimizing the sequence around the PDZ domain. The truncated forms of Nas2 retained their ability to bind to the Rpt5 C-domain and showed only a modest reduction in affinity as measured using bio-layer interferometry (Supplementary Fig. S1b). Nas2LND produced well diffracting crystals after two months, enabling us to determine a structure comprised of amino acids Gln129–Leu220 in the asymmetric unit (Fig. 1). Nas2LND adopts a fold consisting of five β-sheets and two α-helices commonly found amongst PDZ-domain structures (Fig. 1). N-terminal residues from cloning that spanned Gly115–Gly124 could be traced in the electron-density maps (blue in Fig. 1).
However, residues between Gly124 and Gly129 were disordered and could not be modeled. Fig. 1 shows the N-terminal fragment Gly115–Gly124 belonging to the same chain; however, we cannot exclude the possibility it is derived from a neighboring molecule in the crystal.
(Supplementary Fig. S2). Following refinement, recurring electron density was observed in two regions. The first was modeled as a disordered PEG 400 fragment obtained from the cryoprotectant (Supplementary Fig. S3a). The second region was observed between Lys119 and Lys120 and was assigned as a sulfate ion (Supplementary Fig. S3b).

3.2. Structural comparison with other PDZ domains

The structured residues of Nas2 were aligned with the human ortholog p27/PSMD9 (Fig. 2a). Several conserved residues are clustered on the surface of the PDZ domain (Figs. 2b and 2c). Interestingly, there is a group of identical hydrophobic residues covering the region between α-helix 2 and β-sheet 5 (Figs. 2b and 2c), a region that is known to be the peptide-binding groove in other PDZ domains (Lee & Zheng, 2010; Fig. 3a). Similar to what was reported for GRASP55 (PDB entry 3rle), the groove was formed by the final, instead of the second, β-strand (Figs. 3a and 3b), indicating a similar circular permutation from a typical eukaryotic PDZ domain (Truschel et al., 2011). At the entrance of this groove there are conserved charged residues. Besides the groove, there are other surface areas that appear to contain a cluster of conserved residues, maybe facilitating additional binding interactions as the residues do not appear to be structural.

To compare the structure of Nas2 PDZ with a typical PDZ domain that utilizes a GLGF motif for binding the C-terminus of other proteins (Lee & Zheng, 2010), we aligned the structure with PDB entry 1be9 (Doyle et al., 1996). The GLGF motif is located before the β-strand 2 of 1be9. This compares to the region in front of β-strand 5 in Nas2LND owing to the altered β-structure (Fig. S3). Interestingly, a similar motif is found in this region in Nas2LND (GLLG; Figs. 2c–3). However, the role of this sequence in interactions with other proteins is not clear, and the surface areas that appear to contain a cluster of conserved residues, maybe facilitating additional binding interactions as the residues do not appear to be structural.

References