Experimental report

26/11/2017

Proposal: 8-03-884 **Council:** 4/2016

Title: Study on assembly mechanism of 20Sproteasome through the structural analysis of its intermediates

Research area: Biology

This proposal is a new proposal

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Samples: (a4+a5)+(PAC3+PAC4) / protein complex
(a3+a4+a5+a6)+(PAC3+PAC4) / protein complex
(a3+a4+a5)+(PAC3+PAC4) / protein complex
a5 / protein
a5+(PAC3+PAC4) / protein complex
PAC3+PAC4 / protein

Instrument	Requested days	Allocated days	From	To
D22	2	2	16/12/2016	18/12/2016

Abstract:

The 20S proteasome plays a main role, degrading unnecessary proteins, in a protein-metabolic system. This large protein has a hollow cylindrical shape consisting of four rings, a, b, b, a. Both a- and b-rings are heptamers with a1-a7 subunits and b1-b7 ones, respectively. This means that 28 subunits should be timely and precisely arranged to form the active 20S proteasome. It is considered that there are three steps to fabricate the 20S proteasome: the first step is formation of a single a-ring, and the second step is formation of a half proteasome by conjugation of b1-b7 subunits to the single a-ring, and the third step is connection of two half proteasomes. The aim of this study is to reveal the assembly mechanism of a1-a7 subunits in the first step through the clarification of the structure of the intermediates. We have already found several candidates of the intermediates, which are assemblies with a-subunit(s) and chaperones. Because the assemblies have not been crystallized, we will determine the configurations of subunits in the assemblies with small-angle neutron scattering by utilizing contrast matching technique of 75% deuterated protein in 100% D2O solvent.

Experimental Report

Proposal No. 8-03-884
Experiment Team: M. Sugiyama, H. Yagi, M.E.Vigild, A. Martel, L. Porcar, G. Zaccai
Title: Study on assembly mechanism of 20S proteasome through the structural analysis of its intermediates
Instrument: D22, Date of Experiment: from : 16/12/2016To 18/12/2016

[Introduction]

In a protein-metabolic system, the 26S proteasome recognizes and then degrades ubiquitinized proteins. The 26S proteasome is a large protein complex consisting of two regulatory particles and one core particle. The core particle, the 20S proteasome, works as a protease and has a hollow cylindrical shape consisting of four rings, α, β, β, α. The proteolytic sites on the β rings are sequestered within a cavity of the chamber. Substrate entry into this chamber is restricted by a gating pore at the center of the α ring, which opens when protease activators bind to this ring through their C-terminal proteasome-binding motifs Eukaryotic 20S proteasomes are characterized by hetero-heptameric structures of the α and β rings, whose formation is assisted by several proteins termed proteasome assembly chaperones (PACs). By contrast, archaeal 20S proteasomes are composed of a single isoform of an α ring subunit and one or two isoforms of a β ring subunit, which can assemble into a cylindrical four-ring structure without the assistance of proteasome assembly chaperones. Nevertheless, archaeal genomes encode homologs of eukaryotic proteasome assembly chaperones PAC1 and PAC2, which are homologous to each other and function as a heterodimeric chaperone.

The PAC1/PAC2 homologs are named as PbaA and PbaB, respectively. PbaB forms a homo-tetramer and can bind to the proteasome through its C-terminal segment, thereby functioning as a proteasome activator. In contrast, PbaA forms a homo-pentamer and does not interact with the 20S proteasome despite possessing a proteasome-binding motif. However, according to recent structural genomics report, PbaA forms complex with an unknown function protein PF0014 [1]. At first, in order to understand the role of PbaA for assembly mechanism on the 20S proteasome, we analyzed the structure of this unknown complex which is also built by PbaA and PF0014.

The number ratio of PbaA and PF0014 in the complex was found to be 10:10 by native mass spectrometry and Cryo-EM also gave us 3D image of the complex as shown in Fig.1. The next question is the configuration of PbaA and PF0014 in the complex. In this study, we revealed the configuration of PbaA and PF0014 domains in the complex by utilizing inverse Contrast Matching Small-Angle Neutron Scattering (iCM-SANS) method [2].

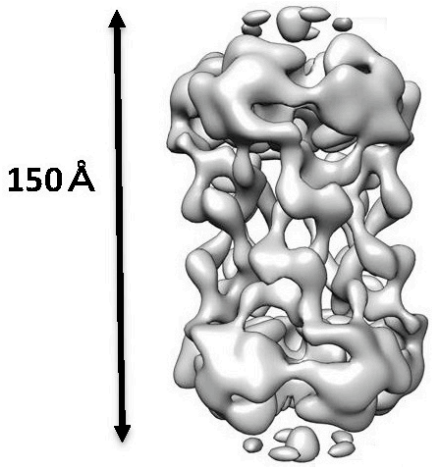

Figure 1. 3D image of PbaA/PF0014 complex by Cryo-EM.

[Experimental]

We prepared for four complex samples in D_2O solution: 75% deuterated (75d) PbaA and hydrogenated (h) PF0014 complex, h-PbaA and 75d-PF0014 complex, h-PbaA and h-PF0014 complex, and 75d-PbaA and 75d-PF0014 complex. Because scattering length density of 75% deuterated protein is matched with that of D_2O, we selectively observe the structure of hydrogenated one in the complex with 75d- and h- proteins in D_2O solution.

Prior to SANS experiments, we performed size-exclusion small-angle x-ray scattering (SEC=SAXS) experiment to confirm the overall structure of the complex in the solution.

[Results and Discussion]

As shown in Fig.2 (a) and (b), we measured the SAXS in the main peak area of SEC chart. Then, we built ab initio 3D-modeling with DAMMIN [3]. Figure 2(c) (cyon) is the obtained structure, which was well consistent with that by Cryo-EM. Figure 2(e) shows the matching quality of our 75% deuteration sample (75d-PbaA and 75d-PF0014 complex), indicating that the 75d-proteins were invisible in D_2O solution. Figure 2(d) shows SANS profile of h-PbaA and 75d-PF0014 complex in which only PbaA domains are visible. The feature of profile is the bump peak around 0.065 $Å^{-1}$, corresponding to the distance ≈100Å. In addition, there was

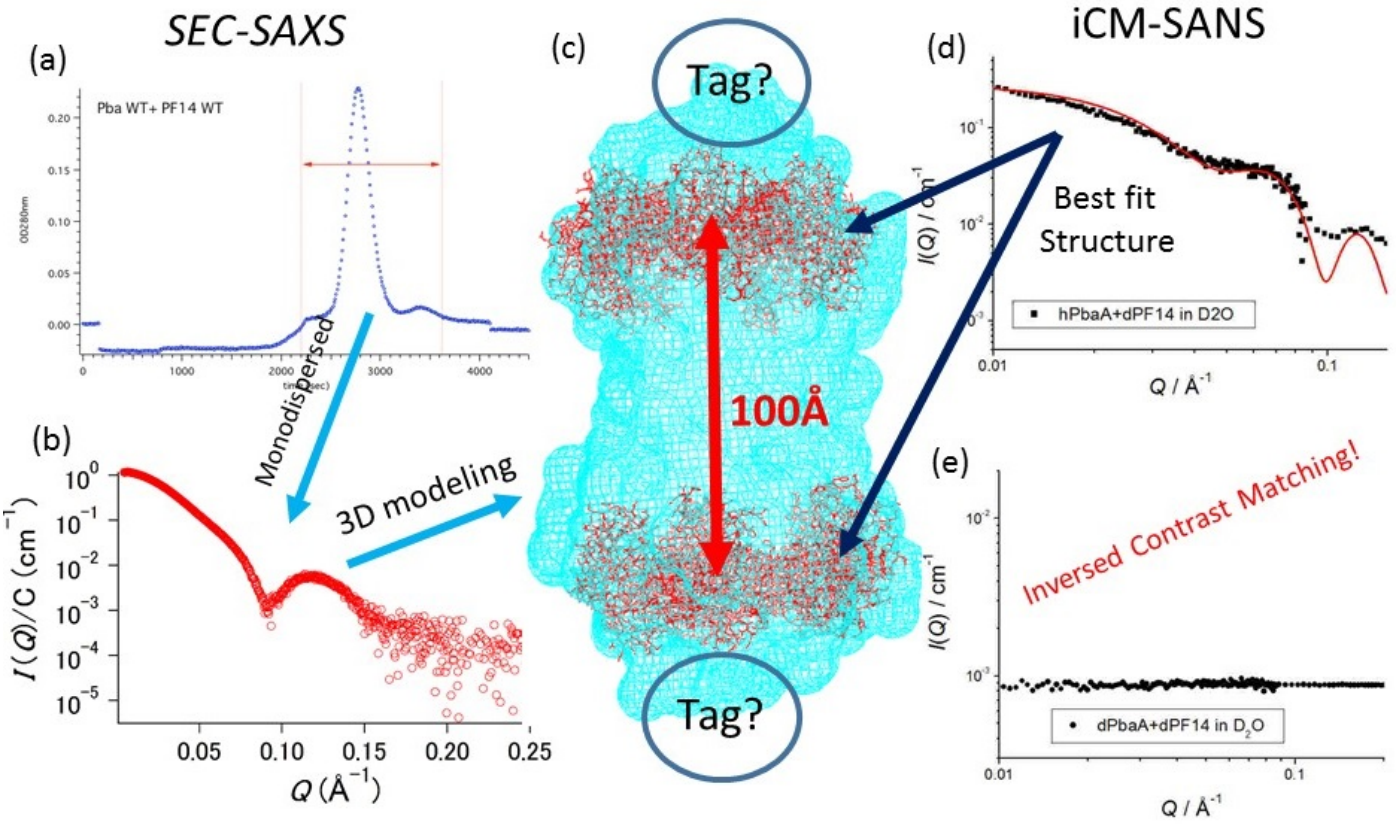

Figure 2. Structural analysis of PbaA and PF0014 complex utilizing with SEC-SAXS and iCM-SANS. (a)SEC- chart. (b) SAX profile (c) 3D model. Cyon region shows overall structure analyzed by SEC-SAXS and ab-initio modeling software, DAMMIN [3]. Red region shows two pentamer PbaA domains which reproduce SANS profile in (d). (d) SANS profile of h-PbaA and 75d-PF0014 complex. Closed circles are experimental data and a red line shows the calculated one with the model displayed in (c) with red regions. (e) SANS profile of 75d PbaA and 75d PF0014 complex.

no peak in the profile of 75d-PbaA and h-PF0014 complex. Therefore, it is rational that the peak corresponds to the distance between two pentamers of PbaA. Based on this idea and overall 3D models obtained by SEC-SAXS, we imposed two pentamers of PbaA with distance of 100Å (Fig.2(c) red). In addition, we also calculated the SANS profile as shown with a red line, which well reproduced the experimental SANS profile. From this, we concluded that, in the PbaA and PF0014 complex, center and outer domains consists of PF0014 and PbaA, respectively.

Now, we are investigating the role of this complex in the assembly mechanism of 20S proteasome.

[References]

[1] An Archaeal Homolog of Proteasome Assembly Factor Functions as a Proteasome Activator: K. Kumoi, et al., *PLos One*, **8** (2013) e60294.

[2] Conformational characterization of a protein complex involving intrinsically disordered protein by small-angle neutron scattering using the inverse contrast matching method: a case study of interaction between α-synuclein and PbaB tetramer as a model chaperone: M. Sugiyama, et al., *J. Appl. Cryst.* **47** (2014) 430–435.

[3] Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing: D. I. Svergun, *Biophys J.* **76** (1999) 2879-2886.