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Complex Formation by the 20S proteasome and Its Activators in an Aqueous Solution

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Introduction

It is interesting of the formation mechanism and the functions of proteasomal systems consisting of several proteins, of which combinations depend upon their functions. The main component in the system is the 20S proteasome (PRS) with the molecular mass of ~700kDa known as a protein degradation machinery in cells. PRS has a hollow cylindrical shape constructed with four rings α-β-β-α rings, each of which consists of seven kinds of subunits. The function of this huge protease complex is regulated through the attachment of other protein complexes termed proteasome activators (PAs): PAs identify proteins which are degraded by PRS. For example, proteasome activator 28 (PA28) with the molecular mass of ~200 kDa has a truncated cone shape consisting of highly homologous α- and β-subunits. It is considered that PA28 connects to both basal planes of PRS and this protease complex (PRS + two PA28; PRS2PA28) contributes to the processing of antigenic proteins into peptides for presentation via the MHC class I pathway. Therefore, PA28, PRS and PRS2PA28 are very important in our immune system.

For a better understanding of the assembly mechanisms in this proteasomal system, it is necessary to reveal the state of PA28, PRS and PRS2PA28 in an aqueous solution. Therefore, we have started to characterize the state of components in the proteasome system. In our previous study, it was clarifies that two PA28s are in dissociation equilibrium. In this study, we examined the formation of PRS2PA28 in a solution of PA28 mixtured with PRS by utilizing small-angle scattering.

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Experimental

The PA28 α-subunit lacking the loop segment (residue Val69 to Lys97) was generated using standard PCR and genetic-engineering techniques. A mutated α-subunit and a wild-type β-subunit were subcloned into the expression vectors pET21d and pET23a, respectively. Each subunit was separately expressed using *Escherichia coli* (BL-21) and purified by ammonium sulfate precipitation, hydroxyapatite chromatography, and ion-exchange chromatography. The hetero-oligomer of PA28 was constructed by mixing the two subunits and purified by gel filtration chromatography. PA28 was concentrated to 5.0 mg/mL in a buffer solution composed of the following: 50 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol,

Cells of PRS were grown to an absorbance of 600 nm at 2.0 in YPD medium at 27C. The grown cells were harvested and suspended in 50mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl and 10\% glycerol, then lysed by mixing with glass beads.The extract was centrifuged at 26,740 g for 30 min at 4 C, then the supernatant was subjected to the affinity chromatography using anti-FLAG M2-agarose beads (Sigma). Anti-FLAG M2-agarose beads, to which the 26S proteasome had been bound, were incubated with 50mM Tris-HCl buffer (pH 7.5) containing 500mM NaCl, 10% glycerol and 10% Triton X-100 for 30 min at 25C to dissociate the 19S components from the 26S proteasome. After washing the column, PRS was eluted with 3×FLAG peptide and the concentration was tuned to be 3.8 mg/mL in same buffer.

In order to examine the formation of PRS2PA28 in a aqueous solution, both prepared samples were mixed with the molar ratio of [PA28]:[PRS]=2.6:1.The concentration of the mixture sample was also tuned to be 4.1 mg/mL.

Results and discussion

Figure 1 shows Guinier plot of the mixture of Pa28 and PRS. From this data, the gyration radius was found to be 87Å. The calculated gyration radius of PRS2PA28from PDB data is 86Å. Therefore, our data clearly show that the PA28 and PRS spontaneously form the complex in an aqueous solution.

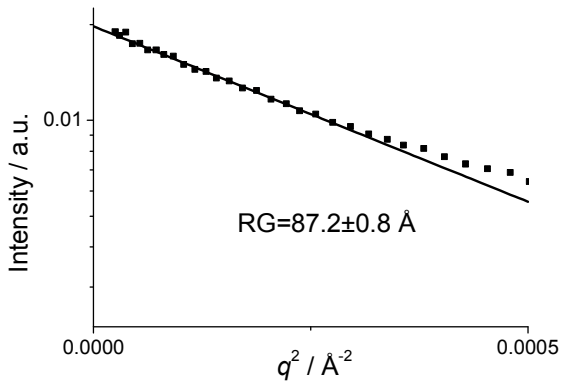

Figure 1. Guinier plot of the mixture of Pa28 and PRS. The straight line indicates the result of the least square fitting with Guinier formula.

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