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Complex Formation Process between the 20S proteasome and Its Activators

Masaaki SUGIYAMA*[1], Hirokazu Yagi[2] and Koichi Kato[2,3]
[1]Research Reactor Institute, Kyoto University, Kumatori, Osaka 590-0494, Japan
[2]Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya 467-8603, Japan
[3]Okazaki Institute for Integrative Bioscience and Institute for Molecular Science,
National Institutes of Natural Sciences, Okazaki 444-8787, Japan

Introduction

Proteasomal system plays an important role in the metabolism of protein. The system mainly consists of the 20S proteasome and its activator. Therefore, it is interesting of the formation mechanism and the functionalization of the complex. 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. Recently, new PA for archaeal proteasome, PbaB, has been found and structurally analyzed [1]. However, it is not clear that the structure of the complex consisting of the 20S archaeal PRS and PbaB: for example, how many PbaBs connect to the the 20S archaeal PRS and where they connect? To answer these fundamental questions, we employed small-angle x-ray scattering to analyze the complex structure in the mixture solution of the 20S archaeal PRS and PbaB.

Experimental

The sample preparation is described in the previous report [1].

In order to examine the number of the connecting PbaB, we prepared for five mixture solution with different [PbaB]:[PRS]; 1:4, 2;4, 4:4, 8:4 and 10:4. The SAXS measurements were performed with SAXSE installed at BL10C of IMMS at KEK-PF. The observed scattering intensities were corrected for background, cell, and buffer scatterings and trasmissions.

Results and discussion

Figure 1 shows SAXS profiles of the mixture of the 20S archaeal PRS and PbaB. From this data, it is clear that, depending upon the ratio of [PbaB]:[PRS], the scattering profiles also has been changed. To make them clearer, the dependence of the gyration radius upon the ratio of [PbaB]:[PRS] was shown in Fig. 2. By increasing the ratio of [PbaB]:[PRS], the value of gyration radius also increased. This means that all PbaBs in the mixture solution make the complex with PRS and then the observed Rg is getting larger because it is an average between the Rg of complexes and that of the remaining PRS. In addition, when the ratio gets over than 2.0, the increase of RG value were ended. This means that all PRSs make the complex with PbaB and the excess PbaBs remain as solo molecules. From this data, it is supposed that two of PbaB connects to the 20S archaeal PRS.

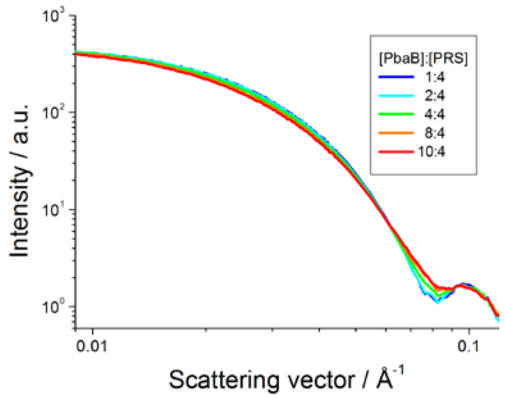

Figure 1.SAXSprofiles with the different ratios of [PbaB]:[PRS].

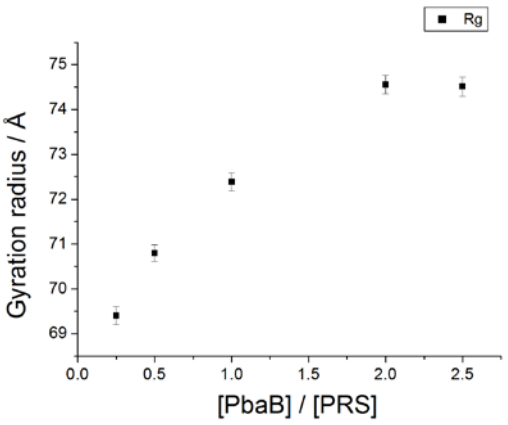

Figure 2. Dependence of gyration radii.

[1] Kumoi K1 Satoh T, Murata K, Hiromoto T, Mizushima T, Kamiya Y, Noda M, Uchiyama S, Yagi H, Kato K, PLoS One. 2013;8(3):e60294.

* sugiyama@rri.kyoto-u.ac.jp