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A small angle X-ray scattering study of ClpA and ClpP from *Escherichia coli*

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Introduction

ATP-dependent proteases play a major role to maintain proper protein homeostasis, contributing to protein quality control and modulating the intracellular concentration of important global regulatory proteins. *Escherichia coli* has several soluble ATP-dependent proteases, Lon, ClpAP and ClpXP. ClpAP degrades *E. coli* denaturated L-glutamate dehydrogenase (GDH) to maintain the cell growth at starved condition [1]. ClpAP is a complex of two heterologous proteins, ClpA, which catalyzes the ATPase activity, and ClpP, which contains the proteolytic active site. Molecular properties of ClpA and ClpP have been studied by hydrostatic methods [2] and electron microscopy [3]. ClpA made a hexameric structure that had Mr 505k in the presence of ATP and ATP analogs. ClpP had subunits arranged in double rings of seven-subunit rings [2,3] that had molecular weights of 300k. Tetradecamer of ClpP was determined only by samall angel X-ray scattering [4]. We try to show that the structure of *E. coli* monomer or dimmer and hexamer of ClpA and heptamer and teteradecamer of ClpP in various solutions using small angel X-ray scattering (SAXS) method.

Materials and Methods

ClpA and ClpP were purified separately as described briefly. ClpA was overexpressed in mostly soluble form in *E. coli* CSH 100 under very strong promoters p_{tac} on multicopy plasmids pSK-39. ClpA was purified as a mixture of monomeric and dimeric forms in 50 mM Tris/pH7.5 /1 mM EDTA/10% (v/v) glycerol /0.2 M KCl. ClpP has been cloned under the control of T7 promoter in a pET3a plasmid and expressed in *E. coli* strain SG1147 clpA::kan. ClpP can be overproduced in a soluble form [5]. Purified ClpP was associated tetradecamer in 50 mM Tris-HCl, pH 7.5, containing 0.2 M KCl .

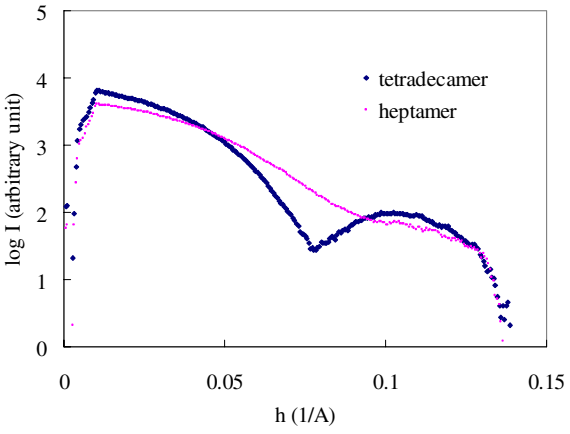

Figure Scattering patterns of ClpP tetradecamer and heptamer

Small-angle X-ray scattering (SAXS) experiments were performed at BL-15A1. The sample solution was irradiated with monochromatic X-rays (1.504 A) and scattered X-ray intensities were recorded on a two-dimensional CCD-based X-ray detector with a camera length of 2342 mm and channel width of 0.307 mm. The raw SAXS data were corrected for intrinsic image distortion, non-uniformity of response and contrast reduction. After image distortion correction, the 2-dimensional data were translated to 1-dimensional data (512channels) using circular averaging.

Results and Discussion

ClpA

Rg of ClpA showed 63 Å in the presence of 2 mM ATPγS. There were no subpeaks in the scattering pattern of ClpA. It seems that ClpA structure would not have one rigid structure. One may not get a clear data similar to electron microscopy results of Kessel, M. *et al*.,[3]

ClpP

When ClpP was present with >0.1 M KCl at pH 7.5 at room temperature, ClpP was tetradecamer. After ClpP was put into 20 mM sodium phosphate, pH 7.2, with 0.1 M sodium sulfate at 5 C, ClpP was dissociated into heptamer. Rg values of tetradecamer and heptamer were 45 and 36 Å respectively. The cube of ratio of Rg was 2:1, directly proportional to the molecular weight.

The results of these ClpPs scattering patterns are shown in the Figure. The tetradecamer of ClpP has some ordered structures at inside of ClpP but ClpA did not show any ordered structures.

References

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