15A/2000G325

Nucleotide-induced Structural Changes of GroEL Studied by X-ray Scattering

Tomonao INOBE[1], Munehito ARAI[1], Kazuki ITO[2], Masaharu NAKAO[1], Kiyoto KAMAGATA[1], Yoshiyuki AMEMIYA[3], Hiroshi KIHARA[4], and Kunihiro KUWAJIMA*[1]

[1]Dept. Physics, Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan [2]Inst. Materials Sci., Univ. Tsukuba, Tennodai, Tshukuba, Ibaraki 305-8572, Japan [3]Dept. Adv. Materials Sci., Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan [4]Dept. Physics, Kansai Med. Univ., Uyamahigashi, Hirakata, Osaka 573-1136, Japan

INTRODUCTION

Escherichia coli chaperonin GroEL assists correct folding of a newly synthesized polypeptide *in vivo*. GroEL is a tetradecameric protein of 14 identical 57 kDa subunits arranged in two heptameric rings stacked back-to-back with a central cavity. Chaperonin-assisted protein folding proceeds through cycles of ATP binding and hydrolysis by GroEL, which undergoes a large structural change by the ATP binding and hydrolysis. In order to elucidate the GroEL structural chages in solution during ATPase cycle, the small angle X-ray scattering (SAXS) patterns of GroEL were measured with a two-dimensional CCD-based X-ray detector, and compared with SAXS patterns calculated from the three-dimensional structures determined by X-ray crystallography.

EXPERIMENTAL

GroEL was overexpressed in *E. coli* and purified. Buffer for GroEL samples contained 50 mM Tris-HCl (pH 7.5), 100 mM KCl, and 10 mM $MgCl_2$. Scattering patterns were recorded by a CCD-based X-ray detector. The scattering patterns of GroEL were measured in the presence and absence of 2 to 5 mM nucleotide. The concentration of GroEL was 3 to 10 mg/ml, and accumulation time of the scattering data was 20 to 85 sec. The raw SAXS data were corrected for intrinsic image distortion, non-uniformity of response and contrast reduction. Theoretical SAXS patterns were calculated from the coordinates of crystallographic structures with non-resolved amino acids placed in bottom of the cavity using the program CRYSOL, which include a single hydration layer of density 0.334 $e^-/Å^3$ on the surface of the molecule.

RESULTS AND DISCUSSION

Figure 1A and B represent scattering patterns of GroEL with and without ATP. In the absence of the nucleotide, the GroEL scattering pattern measured in solution displays three major maxima at Q = 0.07, 0.125 and 0.17 $Å^{-1}$ (bold line in Fig. 1A). In the presence of ATP, the scattering pattern has distinct two maxima (Q = 0.07 and 0.14 $Å^{-1}$) (bold line in Fig. 1B). Each peak and trough shift toward higher Q region than those of unliganded GroEL. This result indicates that ATP induces structural transition of GroEL. Such an ATP-induced structural change of GroEL was also observed in the presence of metallo-fluoride(AlF_4, BeF_x, or GaF_x)-ADP complex, but not observed in ADP, non-hydrolysable ATP analog (ATPγS), nor hydrolysable CTP (data not shown). These results, together with results of biochemical experiments, indicate that the binding of ATP is responsible for the structural change of GroEL and that the energy of ATP hydrolysis is not required for the structural change of GroEL.

Next, in order to gain detailed information about the GroEL structure during the ATP hydrolysis cycle, the scattering patterns calculated from atomic coordinates determined by X-ray crystallography (broken lines in Fig. 1A and Fig. 1B) were compared with the experimental patterns. In the absence of nucleotide, the calculated pattern based on unliganded crystal structure (Fig. 1C) and experimental one are well fitted each other. In this state, GroEL takes a "closed state" with high affinity for non-native proteins in both rings. In the presence of ATP, the scattering pattern was calculated from the GroEL portion of atomic coordinates of the GroEL-GroES-ADP complex (Fig. 1D). It agreed well with the experimental pattern, indicating that GroEL takes an "open state" which has an increased volume cavity owing to an upward movement of apical domains.

Consequently, it is concluded that GroEL alternates its structure (open < close) in a concerted manner during the ATP hydrolysis cycles.

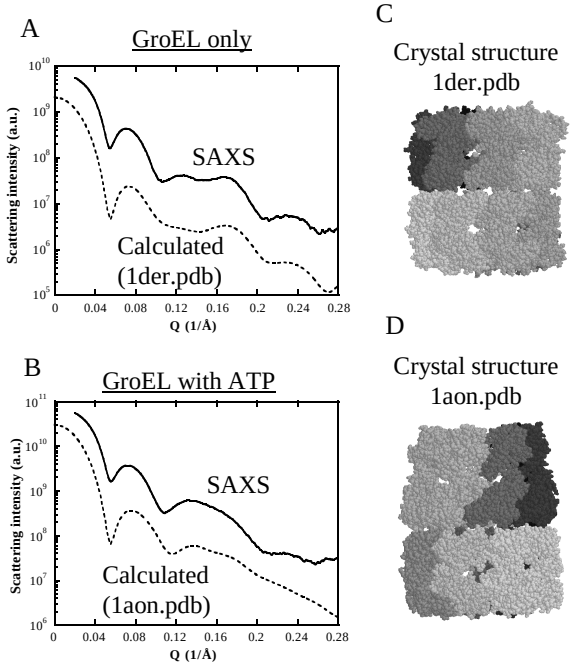

Figure 1. Experimental and calculated X-ray scattering pattern of GroEL

*kuwajima@phys.s.u-tokyo.ac.jp