ADP and ATP Destabilize the *Escherichia coli* Chaperonin GroEL whereas Potassium Ion Does Not; Structural Evidence by the Solution Small Angle X-ray Scattering Measurements

Yuzuru Hiragi and Kaoru Ichimura

ATP, ADP and Mg^{2+} dependence of the denaturation by Guanidine Hydrochloride of the GroEL, a chaperonin originated from *Escherichia coli*, was investigated by the solution small-angle X-ray scattering (SAXS) measurements using synchrotron radiation source. Disappearance of the quaternary structure of the GroEL was observed by the Kratky plots of the scattered intensities. Disintegration of the quarternary structures occurred at Guanidine Hydrochloride (GdnHCl) concentration of 0.8M in the presence of Mg-ATP, 1.1M for Mg-ADP and 1.3M for K^+; decreasing curves of weight averaged molecular weights observed from forward scatterings are shown to be compatible with this results. The destabilization of GroEL by nucleotides was proved structurally.

Key words ; Small-angle X-ray scattering/ GroEL / GdnHCl denaturation / Nucleotides

E. coli GroEL is a member of chaperonin, a family of molecular chaperones which assist refolding of nonnative proteins. It consists of 14 subunits of molecular weight *ca*. 57,000 dalton. Seven subunits of the tetradecamer are arranged to form a ring. Two of the 7 member rings contact each other in back to back manner completing whole GroEL oligomer. Hydrolysis of adenosine triphosphate is required to assist the refolding of the nonnative substrate proteins.

Apart from the refolding mechanism of the substrate proteins, dissociation and reconstitution mechanism of GroEL itself is interesting in the scope of the constitution principle of the oligomeric proteins by estimating from their structural stability. In the case of denaturation by guanidine hydrochloride (GdnHCl), magnesium ion stabilizes GroEL, whereas magnesium-adenosine diphosphate (Mg-ADP) and triphosphate (Mg-ATP) destabilizes. We studied the structural change during the denaturation of GroEL by the method of solution small-angle scattering method (SAXS).

SAXS experiments were carried out at 25˚C with the optics and detector system of SAXES installed at the 2.5 GeV storage ring in the Photon Factory, KEK,Tsukuba, Japan. Scattering intensities at wave length of 1.49Å were registered in the range $0.013\ Å^{-1} < Q < 0.355\ Å^{-1}$, where Q denotes the amplitude of the scattering vector equal to $4\pi \sin(\theta/\lambda)$ and 2θ is the scattering angle. Specimen chamber was kept at 25 ˚C throughout the

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Scope of research

Our research aims are to elucidate structure-function relationships of biological macromolecules, mainly proteins, by using physicochemical methods such as spectroscopic and X-ray diffraction methods. The following attempts have been mainly made in our laboratory for that purpose. (1) X-ray diffraction studies on protein structures in crystal and in solution are carried out by crystallographic and/or small-angle X-ray scattering techniques to elucidate structure-function relationships of proteins. (2) Molecular mechanism for myosin assembly is studied by proteolytic method, electron microscopy, and computer analysis of the amino acid sequence.

Assoc Prof
HATA,
Yasuo
(D Sc)

Instr
HIRAGI,
Yuzuru
(D Sc)

Instr
FUJII,
Tomomi
(D Sc)

Instr
AKUTAGAWA,
Tohru

Students:
HIDA, Kouichi (RF, D Sc)
KURIHARA, Eiji (MC)
SAKAI, Hisanobu (MC)
HASEGAWA, Junya (MC)

experiments. Net scattering intensities were calculated by subtracting the scattering intensities of a blank buffer solution from those of the assembly solution. Absorption of X-ray by chloride atoms was corrected.

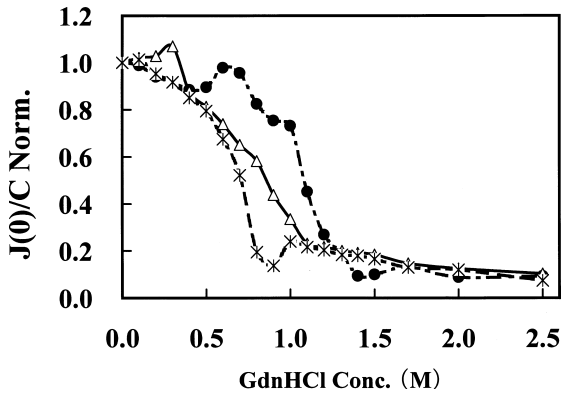

Figure 1. Change of *J(0)/C*, proportional to the weight average molecular weight against GdnHCl concentration; addition of potassium ion only (•), Mg-ADP (Δ), and Mg-ATP (*).

Fig. 1 shows the change of *J(0)/C*, against GdnHCl concentration with the addition of potassium ion only, Mg-ADP, and Mg-ATP. *J(0)* is scattered intensity extrapolated to scattering vector Q=0 and C is the concentration of the protein and is proportional to the weight average molecular weight. In the case of the process of the structural change, what observed are the mixture of intact and denatured particles. Consequently solution system is polydisperse. Molecular weights obtained here are, therefore, weight average molecular weight.

In the absence of nucleotides, decrease in the molecular weight proceeds slowly. When nucleotides were added, the denaturation was accelerated in both case. ATP seems to destabilize stronger than ADP. Probably hydration of ATP during measurements affected the protein stability.

Disappearance of quaternary structure is impossible to detect from the average molecular weight. This is easily observable by the Kratky plots, $J(Q)*Q^2$ *vs* Q, of the scattered curves. Fig. 2 shows the plots in different solution conditions. The first peak in low Q region is typical for globular proteins. The second peak around Q=0.075Å^{-1} derives from the proteins having the quaternary structure. As can be seen from Fig2. (a), the second peak together with first peak, disappeared at the GdnHCl concentration of 1.3M for potassium ion solution. Likewise the peaks did at 1.1M and 0.8M GdnHCl concentration in the presence of Mg-ADP (Fig.2 (b)) and ATP (Fig.2. (c)), respectively. As the first and the second peaks disappeared simultaneously, GroEL denatured directly to unfolded coil without passing the globular monomer structure in the equilibrium denaturation.

In the addition of ADP or ATP decrease in average molecular weight and disappearance of Kratky peaks proceeds in lower GdnHCl concentration. This is interpretable by the effects of nucleotides on the inter-subunit interaction of GroEL. Under the existence of nucleotides, large movement of apical domain is expectable even when GroES, the helper of chaperonin, does not exist. Increase in the flexibility in the subunit would contribute to the fragileness of the GroEL.

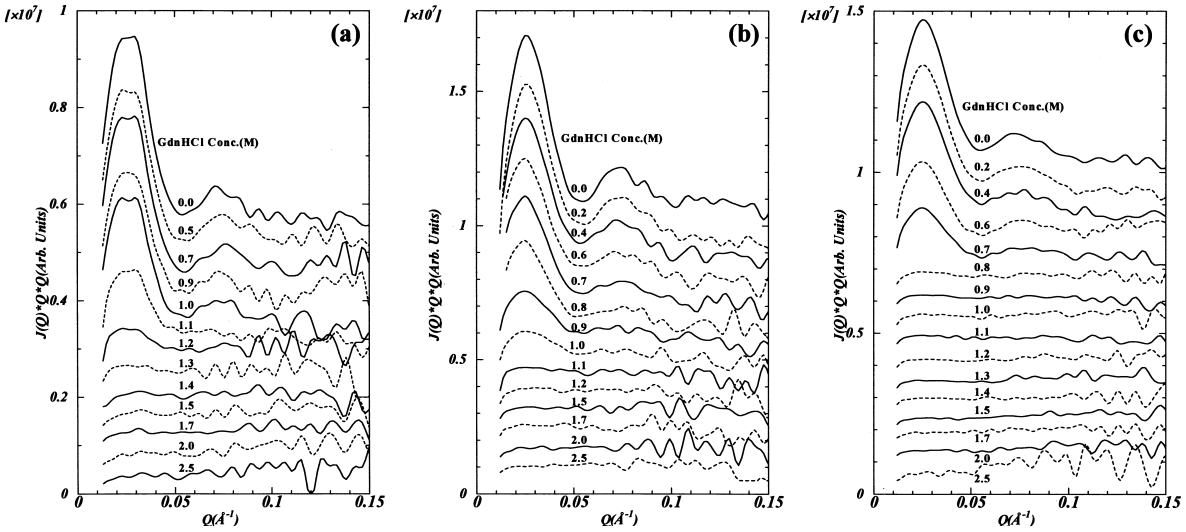

Figure 2. Change of Karatky plot patterns against GdnHCl concentration; (a):addition of potassium ion, (b):Mg-ADP., and (c):Mg-ATP.