# GroES co-chaperonin small-angle X-ray scattering study shows ring orifice increase in solution

A.A. Timchenko[a], B.S. Melnik[a], H. Kihara[b], K. Kimura[c], G.V. Semisotnov[a,*]

[a] *Institute of Protein Research, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia*
[b] *Physics Laboratory, Kansai Medical University, Uymahigashi, Hirakata 573, Japan*
[c] *Dokkyo University, School of Medicine, Mibu, Tochigi 321-02, Japan*

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**Abstract GroES consists of seven identical 10 kDa subunits and is involved in assisting protein folding as the partner of another oligomeric protein, the GroEL chaperonin. Here we studied the GroES structure in solution using small-angle X-ray scattering (SAXS). The SAXS pattern, calculated for the GroES crystal structure, was found to be different from the experimental one measured in solution. The synchronic shift in the radial direction and some turning of the protein subunits eliminate the difference and result in the increase of the hole diameter in the GroES ring-like structure from 8 Å in the crystal to 21 Å in solution.**



*Key words:* Small-angle scattering; Chaperonin; GroES

## 1. Introduction

*Escherichia coli* cells contain two major oligomeric proteins, GroEL and GroES, whose synthesis essentially increases at various cell stresses [1,2]. The GroEL (chaperonin) was shown to be involved in protein folding and translocation through membranes [3,4], interacting with non-native protein chains [5]. The GroES (co-chaperonin) in its turn tightly interacts with GroEL in the presence of ATP or ADP [6] affecting the structure [7,8] and stability [9] of the GroEL particle and together with ATP or ADP decreasing the interaction of GroEL with non-native protein targets [10,11].

The spatial structures of GroES and GroEL alone and their complex in the presence of ADP have been established by X-ray crystallography [8,12–14]. GroES consists of seven identical 10 kDa subunits arranged in the ring-like oligomeric particle described as a dome 30 Å in height and 70 Å in diameter with a 8 Å wide central hole in the dome roof [8,14]. The GroES heptameric ring interacts with one of two heptameric rings of the GroEL chaperonin forming a stable asymmetric complex [6–8]. The GroES structure is little changed upon binding with GroEL (only the extended mobile loop of each GroES subunit is fixed in the complex with GroEL) [8,14]. Despite the extensive crystallographic structural information for GroES and GroEL [8,12–14], the mechanism of the chaperonin-assisted protein folding is still not understood. One of the intriguing features is the conformational plasticity of the GroEL and GroES oligomeric particles in solution that may be an important regulator of the chaperonin-mediated protein folding reaction cycle. The crystallographic study of the GroEL·GroES complex shows that the interaction of GroEL with GroES is accompanied by large-scale interdomain movements in GroEL [8,12,13] confirming the high conformational flexibility of the GroEL structure. The high flexibility of the GroES oligomeric structure was also proposed on the basis of the protein crystal structure analysis showing poor intersubunit contacts [14].

In the present work the GroES structure in solution has been studied by small-angle X-ray scattering (SAXS). The SAXS pattern, calculated on the basis of the GroES crystal structure atomic coordinates, markedly differs from the experimental one measured in solution. The GroES crystal structure was modified to achieve the coincidence of the calculated SAXS pattern with the experimental one. The model GroES structure in solution is different from the crystal one mainly due to the shift in the radial direction and small turning of the protein subunits resulting in ring expansion and more than a 2-fold increase in the size of the central hole in the roof of the dome-like protein structure.

## 2. Materials and methods

### 2.1. GroES

GroES was purified according to the known protocol [15] after expression in *E. coli* cells of the multicopy pGroE4 plasmid (the complete groE operon of *E. coli* cloned in the *Eco*RI site of the pACYC184 vector). The purified protein was characterized by SDS–PAGE and size-exclusion chromatography. The protein concentration was determined spectrophotometrically using $A^{1\%}_{275} = 1.4$ [15].

For SAXS measurements the GroES samples were dissolved in a pH 7.5 buffer containing 20 mM Na-acetate, 100 mM K-acetate, 10 mM triethanolamine acetate, 1 mM EDTA, 1 mM DTT, 10 mM Mg-acetate and bidistilled water. The buffer components were analytical grade from Sigma.

### 2.2. SAXS experiments

SAXS experiments were performed on the synchrotron beam-line 15 Å small-angle installation of the Photon Factory, National Laboratory for High Energy Physics (Tsukuba, Japan) [16]. The protein solution in the mica cell with a 1 mm path length thermostated at 23°C was irradiated with a monochromatic X-ray beam (wavelength 1.5 Å). The scattering intensities were collected with the argon-filled linear detector positioned symmetrically with respect to the incident beam and having 512 channels of 0.368 mm width each. The sample-to-detector distance was 2.35 m giving the range of the scattering

*Corresponding author. Address for express mail: Institute of Protein Research (Moscow office), Room 104, Vavilova Street 34, Moscow, GSP 1, 117334, Russia. Fax: (7)-95-9240493 or (7)-95-135-9984 (Moscow office).
E-mail: bmelnik@alpha.protres.ru

*Abbreviations:* SAXS, small-angle X-ray scattering; $R_g$, radius of gyration


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vector values ($h = 4\pi\sin\theta/L$, where $L$ is the wavelength and $2\theta$ the scattering angle) from 0.002 to 0.15 Å$^{-1}$. The SAXS data were treated with the SAXSTIM program in the usual manner [17,18]. The concentration of GroES was varied in the range of 3–15 mg/ml to control the concentration dependence of the scattering data.

### 2.3. Calculation of the SAXS pattern

Calculation of the SAXS pattern for the GroES crystal structure was performed using the 'cube' method [19] and the protein atomic coordinates [14]. The procedure was modified to permit calculation of the SAXS pattern for large oligomeric proteins. It includes choice of the cube size (in our case the cube edge was 1.2 Å) and calculation of the overall scattering amplitude according to:

$$A(\bar{h}) = \sum_{j=1}^{n} A_j(\bar{h})\exp(i\bar{r}_j) \quad (1)$$

where $A_j(\bar{h})$ is the scattering amplitude of the $j$th subunit and $\bar{r}_j$ is the radius vector of its center of gravity. The resulting scattering intensity was calculated by averaging over the $\bar{h}$ orientation according to:

$$I(\bar{h}) = \langle |A(\bar{h})|^2 \rangle \quad (2)$$

The finding of the protein solution structure implies the modification of the protein crystal structure, the calculation of the SAXS curve for the modified structure (taking into account the non-specific solvent influence on a scattering curve) and its comparison with the experimental SAXS pattern to achieve the best coincidence. In our case the modification consisted in the change of the protein subunit position within the GroES oligomeric particle (the moving apart and turning of each subunit around its center of gravity to maintain the intersubunit interactions) as shown in Fig. 1. To evaluate quantitatively the coincidence of the experimental SAXS pattern with the calculated one we used the $R$-factor which reflects the proximity of the calculated and experimental protein volume values [20,21]:

$$R = \frac{\int_{h_{min}}^{h_{max}} [I_{exp}(h) - kI_{model}(h)]h^2 dh}{\int_{h_{min}}^{h_{max}} I_{exp}(h)h^2 dh} \quad (3)$$

where $k$ is the scale factor and $I_{exp}(h)$ is the experimental SAXS pattern, and $I_{model}(h)$ is the calculated one for the model structure.

## 3. Results and discussion

SAXS is a powerful method to study the protein structure in solution at physiological conditions (for references see [17,20,22]). In the case when the protein crystal structure is established up to its atomic coordinates, SAXS yields more detailed information on the protein structure in solution and on large-scale conformational changes induced by interaction with ligands or other proteins. This information is available by calculating the SAXS pattern using the protein crystal structure data [19–22]. When the SAXS pattern calculated for the protein crystal structure is different from the experimental one, the most appropriated protein structure in solution can be found by modifying the crystal structure to eliminate the difference. Since the SAXS pattern is sensitive mainly to the overall dimensions and shape of a protein molecule [17] the most probable modifications of the protein crystal structure are the changes of positions of the large structural blocks, such as protein domains and subunits [20–22]. The application of such an approach to equine liver alcohol dehydrogenase [22] and ribonuclease A [23] did not show a

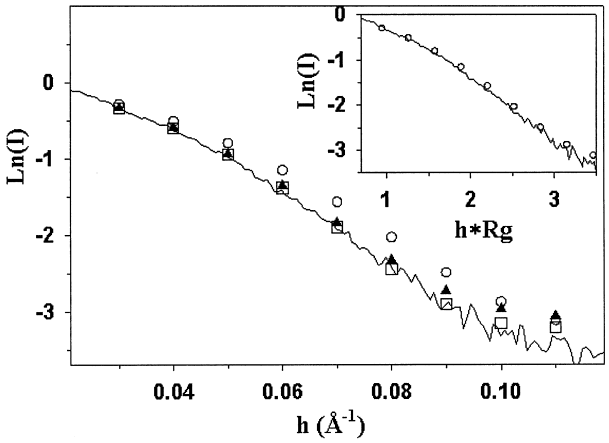


Fig. 2. The SAXS patterns of the GroES oligomeric particle: the experimental one measured at a protein concentration of 7.5 mg/ml (dotted line), calculated from the original crystal structure (○), calculated from the modified crystal structure with uniform ring expansion (▲), and with an additional 15° $x$-turn and a 25° $z$-turn of subunits (□). Inset represents the experimental (dotted line) and calculated for the original protein crystal structure (○) SAXS patterns in the dimensionless coordinates.


Fig. 1. Schematic representation of the GroES crystal structure modification. The protein subunits were moved apart in the direction of the $y$-axis and rotated around the $z$- and $x$-axes. The center of coordinates was placed in the subunit's center of mass.

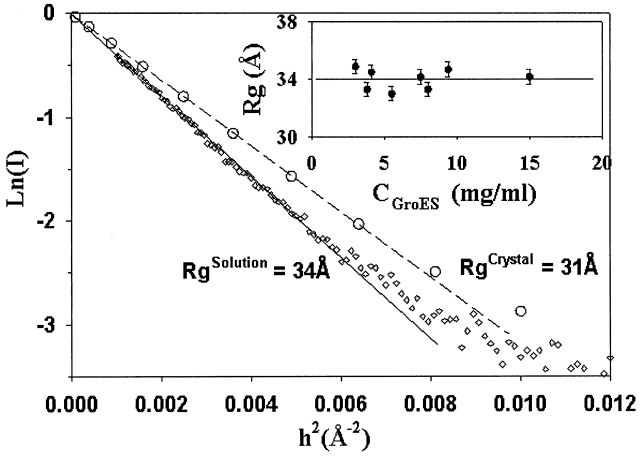


Fig. 3. The Guinier plots of the experimental (◇) and the calculated from the original crystal structure (○) SAXS patterns of GroES. Inset represents the dependence of the experimental $R_g$ value on the protein concentration.

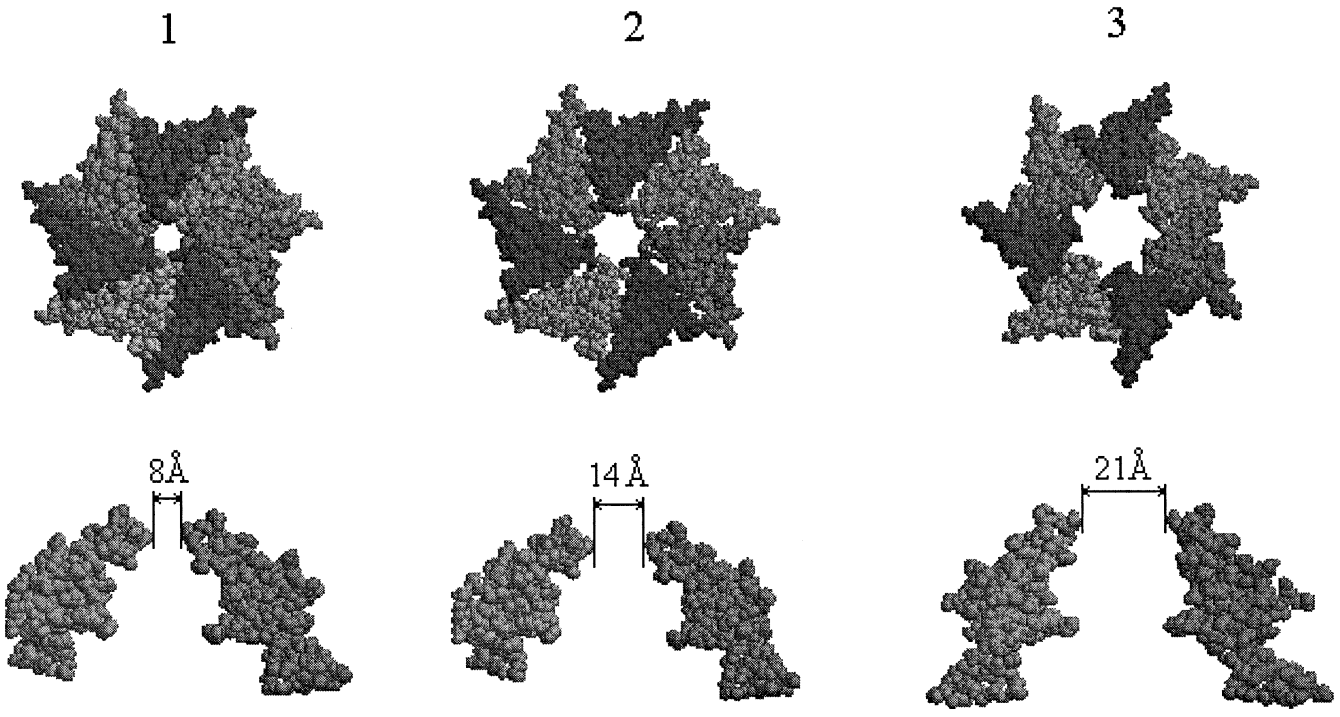


Fig. 4. The GroES original crystal structure [14] (1), the GroES crystal structure modified by a synchronous shift of the protein subunits in the radial direction to provide an $R_g$ value increase of 3 Å (2), and with an additional 15° $x$-turn and a 25° $z$-turn to provide good intersubunit contacts (3). Upper row shows top view of GroES, lower row side view of two opposite subunits.

noticeable difference between the experimental and calculated for the crystal structure SAXS patterns. These results confirm the validity of the procedure based on the detailed description of the protein surface and demonstrate the low sensitivity of the SAXS pattern to block (domain) sliding in contrast to the locking–unlocking movements. The functional domain locking movement was observed for yeast phosphoglycerate kinase [22] where an essential difference between the experimental and calculated for the protein crystal structure SAXS patterns was found.

Fig. 2 represents the GroES SAXS patterns measured in solution and calculated for the protein crystal structure. One can see the essential difference of these SAXS patterns in a wide range of scattering vector values. The difference is mainly due to the change of overall dimensions of the protein molecule in solution. Indeed, the value of the radius of gyration ($R_g$) for the GroES molecule in solution was estimated from the initial part of the scattering curves (Guinier plot [17]) to be $34.0 \pm 0.5$ Å, while the crystal structure shows an $R_g$ value of 31 Å (Fig. 3). It should be noted that the concentration dependence of the $R_g$ value is negligible (Fig. 3, inset) showing that intermolecular interactions cannot be the reason for the increase of the $R_g$ value in solution. Moreover, presentation of the SAXS pattern in dimensionless coordinates $\mathrm{Ln}(I) = f(h \cdot R_g)$ demonstrates the proximity of the SAXS pattern calculated for the protein crystal structure and the experimental one (Fig. 2, inset). This means that the shape of the GroES molecule in solution is similar to that in the crystal.

A simple modification of the GroES crystal structure to increase the $R_g$ value of 3 Å and to maintain its shape is the uniform moving apart of the protein subunits as shown in Fig. 1. The SAXS pattern calculated for this modified crystal structure is very close to the experimental one (Fig. 2). However, this modification leads to weakening of the intersubunit contacts (see Fig. 4). To maintain the intersubunit interactions within the model GroES structure the protein subunits have been turned around their centers of gravity. The criterion for good intersubunit contacts was that the distance between the contacting groups has not exceeded 4 Å. Two possible axes for protein subunit rotation are shown in

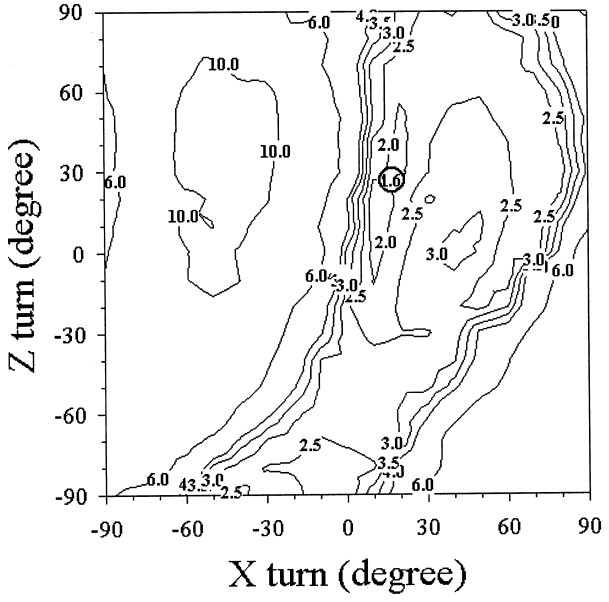


Fig. 5. Dependence of the $R$-factor value (indicated along the lines) on the $x$- and $z$-turns of the GroES subunits in the modified protein crystal structure with a fixed $R_g$ value of 34 Å. The $R$-factor local minimum of 1.6 is indicated by a circle.

Fig. 1. The dependence of the *R*-factor value (see Section 2) on the angles of subunit rotation has been analyzed to choose the angles which provide the best coincidence of the experimental and calculated SAXS patterns and good intersubunit contacts. This dependence is represented in Fig. 5. It is seen that the minimal *R*-factor value is realized at a rotation angle of $15 \pm 5°$ around the *x*-axis and $25 \pm 5°$ around the *z*-axis. The presence of one deep minimum shows that modeling of the GroES solution structure by the above procedure is sufficiently unambiguous. It was ascertained that the changes of position of the smaller parts of the GroES structure (such as the extended mobile loops or subdomains) do not noticeably affect the protein SAXS pattern (not shown). Thus, the final model structure of GroES in solution is the result of the following modification of the protein original crystal structure: (1) the synchronous shift of the protein subunits in the radial direction to provide the increase of the $R_g$ value from 31 Å to 34 Å, and (2) the rotation of each subunit around the *x*-axis of 15° and around the *z*-axis of 25° to provide intersubunit contacts at the increased protein dimensions (Figs. 1 and 4). This final model structure of the GroES molecule in solution has the essential intersubunit contacts (see Fig. 4) and an SAXS pattern which is in good coincidence with the experimental one (see Fig. 2). On the other hand, the modification of the protein crystal structure mentioned above results in an essential increase of the ring orifice of the GroES dome-like oligomeric particle from 8 Å to 21 Å (see Fig. 4).

The high plasticity of the GroES oligomeric structure has been proposed earlier on the basis of X-ray crystallography data analysis [14]. It has been found that the packing interactions between the β-hairpins, which form the roof of the GroES dome, seem to be weak [14]. This observation suggests that the GroES has a metastable oligomeric structure permitting opening of the roof of the GroES dome [14]. The results of the present work confirm that the position of subunits within the GroES oligomeric particle is noticeably changed in solution in comparison with that in the crystal.

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